



UNIVERSITY
OF TASMANIA

**PHASE-SHIFT DYNAMICS AND RESILIENCE OF
TEMPERATE REEF STATES TO URBAN STRESSORS**

by

NINA KRIEGISCH

Diploma in Marine Biology, University of Kiel

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Institute for Marine and Antarctic Studies



University of Tasmania

August 2016

Declarations

Statement of originality

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution. The work contained in this thesis, except where otherwise acknowledged, is the result of my own investigations.

Signed: (Nina Kriegisch)

Date: 08/08/2016

Statement of authority of access

This thesis may be available for loan and limited copying in accordance with the Copyright Act 1968.

Signed: (Nina Kriegisch)

Date: 08/08/2016

Abstract

In recent decades there has been a concerning trend of loss of kelp beds to sea urchin ‘barrens’ as a result of overgrazing by urchins. These two habitat states have been extensively studied across many systems worldwide, however the underlying dynamics of the transition to barrens, the recovery to kelp, and mechanisms of resilience of either habitat configuration are not fully appreciated. This thesis studies the transitions of kelp beds to sea urchin barrens and *vice versa* in relation to the stressors typical of urbanised coastal areas, namely enhanced levels of nutrients and sediment. At several sites in Port Phillip Bay (Victoria, Australia) adjacent the city of Melbourne, the role of sea urchins (*Heliocidaris erythrogramma*) in influencing the abundance of kelp (*Ecklonia radiata*) and other macroalgae was examined by assessing behavioural traits of the urchins and characteristics of both urchins and kelp in experiments considering both the ‘bottom-up’ force of nutrients and the ‘top-down’ force of grazing, together with the effects of sedimentation.

It is revealed that the sea urchin is a major driver of community structure on rocky reefs in Port Phillip Bay. Sediment enhancement has no direct effect on intact kelp beds, while nutrient enhancement can play a beneficial role in strengthening the resilience of kelp beds by stimulating growth of kelp, but grazing of urchins overwhelms the positive effect of the nutrients. Densities exceeding 4 urchins m⁻² increase the risk of overgrazing with a definite phase-shift to urchin barrens at densities of 8 urchins m⁻² or greater. Conversely, kelp bed recovery will only occur when urchin densities are below 4 m⁻². Nutrient enhancement does not change the thresholds in either direction. Assessments of sea urchin behaviour reveal that the availability of drift-kelp alters urchin foraging behaviour, so that if sufficient drift material is available, urchins will not actively overgraze attached algae.

Considering that neither enhancement of nutrients nor sediments changes the likelihood of a phase-shift, sea urchins should be the focal point of any effort to protect existing kelp beds or effect the recovery of kelp on urchin barrens. It is therefore essential to identify the tipping points in urchin biomass/density that lead to shifts in either direction, and how to influence urchin foraging behaviour to prevent overgrazing of attached algae.

Acknowledgements

I am grateful for the institutional support from the Institute for Marine and Antarctic Studies (IMAS) and the University of Tasmania for giving me the opportunity for this PhD and a Tasmanian Graduate Research Scholarship. The thesis was founded by the Victorian Department of Environment, Land Water, and Planning and The Holsworth Wildlife Research Endowment.

Thank you very much to my supervisor team Prof. Craig Johnson and Dr. Scott Ling for their time, patience and guidance. Special thanks goes to Dr. Scott Ling for his additional efforts and help with the field work.

Many thanks also go to Dr. Steve Swearer from the University of Melbourne providing the field work material as well as diving equipment, boats and advice.

And of course, I have to thank my team in the field, mainly Simon Reeves and Dean Chamberlain. This thesis would not be possible without their help. I particularly appreciate Simon Reeves, for the good times, help and support throughout my whole thesis.

Finally, I would like to thank my husband Kai May for his endless support, time, and understanding during the whole process and my family for being there for me when I needed them.

Statement of co-authorship

Chapters 2-5 of this thesis have been prepared as scientific manuscripts for submission to peer-review journals as identified on the title page of each chapter. In all cases the design and implementation of the research, data analysis, interpretation of results and manuscript preparation was the responsibility of the candidate but was carried out in consultation with supervisors and other specialist contributors.

The following people contributed to the publication of work undertaken as part of this thesis:

Candidate: Nina Kriegisch – University of Tasmania, Institute of Marine and Antarctic Studies

Author 2: Simon Reeves – University of Tasmania, Institute of Marine and Antarctic Studies

Author 3: Dr Scott Ling - University of Tasmania, Institute of Marine and Antarctic Studies

Author 4: Prof. Craig Johnson - University of Tasmania, Institute of Marine and Antarctic Studies

Paper 1: Phase-shift dynamics of sea urchin overgrazing on nutrified reefs

Located in chapter 4

Candidate was the primary author and with authors 3 and 4 contributed to the idea and its formalisation. Author 2 and 3 assisted with the development and author 3 and 4 contributed to the refinement and presentation. Author 3 oversaw development of the experimental approach in the field and Author 2 assisted in the field.

Paper 2: Top-down sea urchin overgrazing overwhelms bottom-up nutrient stimulation of urban kelp beds

Located in chapter 3

Candidate was the primary author and with authors 3 and 4 contributed to the idea and its formalisation. Author 2 and 3 assisted with the development and author 3 and 4 contributed to the refinement and presentation. Author 3 oversaw development of the experimental approach in the field and Author 2 assisted in the field.

Paper 3: Drift-kelp suppresses destructive grazing in sea urchins

Located in chapter 5

Candidate was the primary author and with author 3 contributed to the idea and its formalisation. Author 2 and 3 assisted with the development and author 3 and 4 contributed to the refinement and presentation. Author 3 oversaw development of the experimental approach in the field and Author 2 assisted in the field.

Paper 4: Sea urchin control of seaweed communities across a gradient of urban nutrients

Located in chapter 2

Candidate was the primary author with author 3 contributing to the idea and its formalisation. Author 2 and 3 assisted with the development and author 3 and 4 contributed to the refinement and presentation. Author 3 oversaw development of the experimental approach in the field and Author 2 assisted in the field.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

Prof. Craig Johnson
Supervisor
IMAS
University of Tasmania

Prof. Richard Coleman
Head of School
IMAS
University of Tasmania

Date: 01/08/2016

02/08/2016

Table of contents

| | Page |
|--|------|
| Declarations | i |
| Abstract | ii |
| Acknowledgements | iii |
| Statement of co-author contributions | iv |
| Chapter 1: General introduction | |
| Top-down vs. Bottom-up control | 1 |
| A multitude of stressors | 2 |
| Alternative stable states | 3 |
| Resilience | 5 |
| Urchin foraging behaviour | 5 |
| Thesis structure | 6 |
| Chapter 2: Sea urchin control of seaweed communities across a gradient of urban nutrients | |
| Abstract | 9 |
| Introduction | 10 |
| Materials and methods | 11 |
| Results | 20 |
| Discussion | 23 |
| Chapter 3: Top-down sea urchin overgrazing overwhelms bottom-up nutrient stimulation of urban kelp beds | |
| Abstract | 30 |
| Introduction | 31 |
| Materials and methods | 32 |
| Results | 38 |
| Discussion | 45 |

**Chapter 4: Phase-shift dynamics of sea urchin overgrazing
on nutrified reefs**

| | |
|-----------------------|----|
| Abstract | 52 |
| Introduction | 53 |
| Materials and methods | 54 |
| Results | 59 |
| Discussion | 65 |

Chapter 5: Drift-kelp suppresses destructive grazing by sea urchins

| | |
|-----------------------|----|
| Abstract | 73 |
| Introduction | 74 |
| Materials and methods | 75 |
| Results | 84 |
| Discussion | 88 |

Chapter 6: General discussion

| | |
|---|----|
| The effects of multiple stressors on macroalgal dynamics | 94 |
| Effects of urchin abundance and dynamic feeding behaviour | 97 |
| Synthesis | 99 |

| | |
|-------------------|-----|
| References | 107 |
|-------------------|-----|

| | |
|-----------------|-----|
| Appendix | 124 |
|-----------------|-----|

Chapter 1: General introduction

Temperate rocky reefs dominated by canopy-forming macroalgae provide a complex habitat supporting organisms from many different taxa. Canopy-forming macroalgae provide shelter, food, and a unique physical and chemical environment for a myriad of mobile and sessile fauna. Broadly termed kelp beds when the canopy forming species are large brown algae from the class Phaeophyceae (hereby inclusive of mixed beds of laminarian and furoid algae typical in southern Australia), they provide the foundation for one of the most productive and diverse ecosystems on earth, and support numerous commercially exploited organisms (e.g. rock lobster, abalone, sea urchins, finfish and even macroalgae itself) (Boesch 1974, Dayton 1985, Steneck et al. 2002). It is therefore vital to understand kelp bed dynamics to preserve their ecological functioning, and thus ultimately commercial, recreational and broader societal services.

In recent decades, the collapse of kelp beds to impoverished sea urchin barrens has been increasingly reported (Schiel & Foster 1986, Chapman & Johnson 1990, Estes et al. 1998, Harrold et al. 1998, Sala et al. 1998, Pinnegar et al. 2000, Shears & Babcock 2002, Gagnon et al. 2004, Ling 2008, reviewed by Filbee-Dexter & Scheibling 2014, and Ling et al. 2015). This collapse is of significant concern for natural resource managers, yet even though kelp beds and sea urchin barrens have been extensively studied, the underlying dynamics of the transition to barrens, recovery to kelp, and mechanisms of resilience of either habitat configuration are not fully understood. Many different abiotic and biotic factors interact to control the structure and function of these ecosystems, all of which need to be considered to understand the mechanisms of resilience involved in collapse and recovery of this ecosystem.

Top-down *versus* Bottom-up control

Ecosystems are shaped by a complex array of factors, which have often been conceptualised as mutually exclusive ecological paradigms. Two opposing paradigms are “top-down” *versus* “bottom-up” control, whereby ecosystems are structured by the effects of high trophic levels on lower ones or by low trophic levels on high ones respectively (Hairston et al. 1960, Hunter

& Price 1992a). Top-down control is regulated by consumers (usually either primary or secondary) in a food chain or web, whereas bottom-up control is mediated through rates of primary production, influenced by resources such as nutrient availability and sunlight. The effects of these two controlling factors can be complex, even in relatively simple ecosystems. While many ecosystems have been labelled as either top-down or bottom-up systems, modern views have progressed to acknowledge that both mechanisms may operate simultaneously in a system (Ling et al. 2009a), and to focus on understanding the spatial and temporal variability, plus additional processes or 'stressors', in these opposing and interacting trophic controls (Conversi et al. 2015).

A multitude of stressors

Rocky reef systems are frequently subject to seascape level changes as a result of human-derived stressors moderating the relative strengths of top-down and bottom-up control, particularly when situated near highly populated urbanised areas. Anthropogenic impacts such as pollution (Mearns et al. 2011), invasive species (Hewitt et al. 2004, Piazzzi & Ceccherelli 2006), climate change (Hansson 1977, Hiddink & ter Hofstede 2008, Last et al. 2011), fishing (Jackson et al. 2001) and coastal urbanisation (Noriega et al. 2012) can be directly linked to substantial changes in the configuration of reef systems, and ultimately to the goods and services they provide. These stressors can act singly or more commonly are found to co-occur, and they can potentially interact with each other. In cases where multiple stressors interact, four types of interactive effects are possible: (1) additive, i.e. where the total effect is equal to the sum of individual effects; (2) antagonistic, in which the response is less than the sum of separate effects; (3) mutualistic, where the addition of further stressors does not change the overall effect; or (4) synergistic, when the effect is greater than the sum of their individual effects (reviewed by Crain et al. 2008, and Strain et al. 2014). Thus, the interaction between multiple stressors can create complex indirect 'flow-on' effects (Paine et al. 1998), which can be difficult to predict and may be context dependent, i.e. the effect of a particular suite of stressors may differ across different ecosystems. It is therefore important to understand how multiple stressors (e.g. to climatic, harvest-driven or urban stressors) interact to influence ecosystem responses (Ling et al. 2009a, Boyd & Hutchins 2012, Annala et al. 2014).

Macroalgal communities on urbanised coasts are threatened by enhanced sedimentation, increasing nutrient loads (bottom-up forces) and altered herbivory (top-down force) (reviewed by Strain et al. 2014). Sediment and nutrient loading in these systems has increased due to anthropogenic-driven changes to water catchment and run-off processes leading to a dominance of low-lying algal turfs over kelp beds (Airoldi 2003). Altered herbivory is frequently driven by the cascading impacts of overfishing and subsequent predatory release of herbivore populations (Tegner & Dayton 2000, Ling et al. 2009a). The interaction of top-down and bottom-up forces, mediated by human-derived stressors, can lead to a shift in ecosystem state, which can in some cases represent an alternative and stable ecosystem state.

Alternative stable states

The most problematic response of reefs to anthropogenic stressors and disturbances is arguably when there is a shift from ecologically diverse kelp beds to an undesirable ecosystem state that can persist as a stable alternative system state under identical environmental conditions to that which supports kelp beds (Boesch 1974, Filbee-Dexter & Scheibling 2014, Ling et al. 2015). The dynamics of these systems therefore are characterised by hysteresis, so that if a system experiences a ‘pulse’ perturbation beyond a critical threshold (or ‘tipping point’), return to the former state becomes unlikely even with return to ‘normal’ environmental conditions (Walker et al. 1981). The different stable states are maintained by self-stabilising mechanisms, usually involving some form of positive feedback (Walker et al. 1981, Marzloff et al. 2011). Regime shifts with hysteresis are known as discontinuous or catastrophic phase shifts, and are characterised by multiple tipping points (May 1977, Scheffer et al. 2001).

Not all phase shifts are discontinuous; there are several ways by which an ecosystem may change when facing perturbation (Fig 1). The community state can respond linearly with changes in environmental conditions (Fig 1a), the response can be non-linear or abrupt after a tipping-point is reached (Fig 1b), or it can be discontinuous with multiple tipping-points (Fig 1c). By definition, the discontinuous shift reflects the existence of alternative stable states, i.e. where a system can exist in different states under identical environmental conditions (between the tipping points in Fig. 1c). One state persists with changing environmental condition until a threshold is reached after which there is a change to a new,

qualitatively different state. To induce a shift back to the previous state, the conditions have to reverse further than they were at the point of initial collapse until the reverse tipping-point is reached (Scheffer et al. 2001, Scheffer & Carpenter 2003b, Ling et al. 2009a). At this point environmental changes can trigger transition between alternative stable states, also called attractors. These changes can be major perturbations like storms or species invasions, or subtle changes over a longer time frame can also lead to a catastrophic shift, e.g. eutrophication or slight increases in sedimentation or temperature (Scheffer & Carpenter 2003a, Hughes et al. 2013).

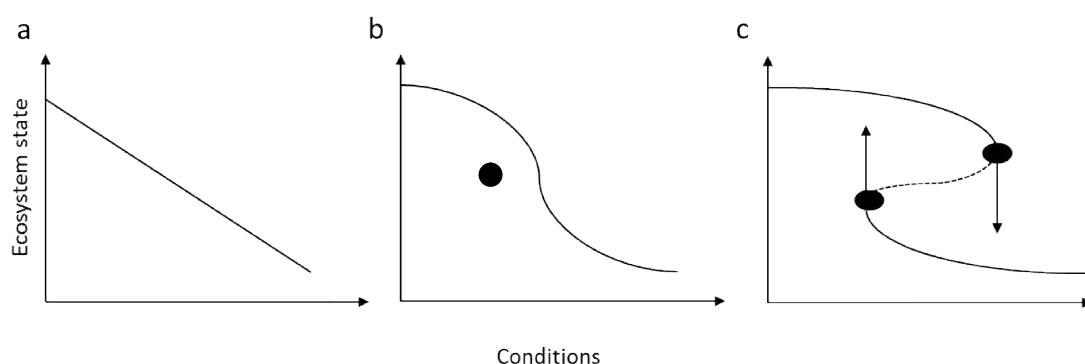


Figure 1. Responses of ecosystem state to changing environmental conditions. a) a smooth linear shift in state with change in environment; b) a more abrupt change in state at a tipping-point (i.e. filled circle); c) an ecosystem with multiple tipping-points and thus alternative stable states (the distance between tipping-points on the x-axis defines the magnitude of the hysteresis of this system); modified from (Scheffer et al. 2001).

In temperate reef environments, perhaps the best studied shift between alternative stable states occurs when rocky reefs undergo phase-shifts from productive kelp beds to impoverished sea urchin ‘barrens’ habitat (Lawrence J. 1975, Chapman 1981, Harrold et al. 1998, Gagnon et al. 2004, Ling 2008). Temperate rocky reef ecosystems change from kelp-dominated to sea urchin barrens when a critical level of grazing intensity is achieved.

While a growing number of examples of how particular single stressors may mediate shifts between these states can be found, there is increasing need to further explore the effects of multiple stressors and their effect on the likelihood of phase-shifts in marine (and other)

systems. It is particularly important to determine how increasing human-derived stressors influence dynamics of subtidal reef ecosystems and the internal mechanisms that provide resilience to these perturbations.

Resilience

Resilience and stability of ecosystems are terms often used to characterise the behaviour of ecosystems subject to perturbation. Definitions of these terms have been debated since the late 1960s, and there continues to be divergent approaches to define them (e.g. Holling 1973, Boesch 1974, Pimm 1984, Tilman & Downing 1994, Holling 1996, Walker et al. 2004). Here, we conform to the definitions of resilience and stability of Holling (1973). Holling describes the concept of resilience as the magnitude to which a system can withstand stress before collapsing or changing into an alternative state, whereas stability refers to the ability of a system to return to its previous (quasi) equilibrium state after perturbation. Holling (1996) later elucidates what he formerly called ‘stability’ as engineering resilience and ‘resilience’ as ecological resilience and suggests that both terms are different aspects of the overall stability of an ecosystem (i.e. nearing equilibrium). Using Holling’s terminology, a system with higher ecological resilience is more capable of withstanding stressors before it undergoes a shift to a different stable state (Peterson et al. 1998). For example, a dense kelp bed with high resilience can experience more grazing pressure before it collapses to an urchin barren. Alternatively, an ecological system with high stability will return quickly to its former state once it has been compromised. In the context of anthropogenic perturbations it is vital to assess the dynamics of both kelp bed collapse and recovery, and to be able estimate potential tipping points associated with sea urchin grazing in this system.

Urchin foraging behaviour

The phase-shift between kelp bed and urchin barren states is understood to be influenced by a multitude of factors, which can be state-dependent (Ling et al. 2015), but an obvious driver is the sea urchin and its *per capita* consumptive effect on kelp beds. Once attaining sufficient local densities and a collective consumptive capacity to overgraze kelp beds, destructive overgrazing may occur (Ling et al. 2010) and can manifest in different ways. The patterns of

grazing, and thus the ultimate impact on local kelp beds, can be influenced by different urchin behavioural modes. Destructive modes include: (1.) highly conspicuous mobile feeding fronts, which involve dense aggregations of sea urchins that destructively graze everything within their path leaving a barren reefscape (Scheibling et al. 1999); or (2.) formation and coalescence of ‘incipient’ barrens created within kelp beds, whereby the kelp bed collapses from the ‘inside out’ with coalescence of smaller barrens patches and the absence of feeding fronts (Flukes et al. 2012). Besides the destructive mode of grazing, sea urchins can also inhabit kelp beds without having a deleterious influence on the standing kelps, even if densities are high (Harrold & Reed 1985, Contreras & Castilla 1987, Vanderklift & Kendrick 2004). In this situation detached macroalgae or ‘drift-kelp’, plays an important role in the diet of sea urchins, and they often remain relatively cryptic whilst feeding on drift material. Moreover, some urchins prefer to feed on drift-kelp (Vanderklift & Kendrick 2005). While the role of food availability has been postulated as a proximate driver of sea urchin behaviour, most studies are observational and critical tests of these putative mechanisms, first raised by Harrold and Reed in 1985, are still lacking.

Thesis Structure

This thesis examines the separate and combined effects of sedimentation and nutrification on patterns of resilience of both macroalgal beds and sea urchin barrens habitat as alternative states of rocky reefs of Port Phillip Bay (PPB), adjacent the city of Melbourne, southeast Australia. The effects of these stressors are considered in the context of impacts of urchin grazing by assessing critical tipping-points of urchin density and biomass, and evaluating urchin behaviour. This is achieved by conducting natural and manipulative experiments within the two community configurations to identify the responses to and relationship between nutrification and sedimentation as urban stressors of reef assemblages. This thesis is laid out as a series of stand-alone manuscripts, therefore repetition of some of the important contextual information within this Chapter (i.e. General Introduction) has been unavoidable.

Chapter 2 introduces the study location in PPB and examines the effects of nutrient gradients near urbanised coastal areas on algal growth when the dominant grazer, the sea urchin *Heliocidaris erythrogramma* is excluded. Chapter 3 displays results of a 3-way factorial experiment in a kelp bed habitat, whereby the single and combined effects of urchin grazing,

nutrient and sediment enhancement were examined simultaneously. This chapter revealed that the major driver in this system is the destructive grazing of *H. erythrogramma* when it is present in high abundance. With this information, another experiment was conducted assessing tipping points in urchin densities in kelp bed and urchin barrens habitat, and the role nutrients play in shifting potential thresholds of overgrazing and recovery. Because of its important role as a driver of the rocky reef systems in PPB, plus its behavioural plasticity from inconspicuously inhabiting kelp beds to destructive overgrazing, a set of experiments were conducted to explore the different feeding modes of this urchin. These experiments assess feeding behaviour of *H. erythrogramma* in kelp bed and barrens habitat, and are detailed in Chapter 5. Chapter 6 is a General Discussion which provides an overview and integration of the results of the 4 ‘data’ chapters (Chapter 2 – 5).

Chapter 2: Sea urchin control of seaweed communities across a gradient of urban nutrients

Abstract

A suite of bottom-up ‘resource-driven’ and top-down ‘consumer-driven’ forces interact to shape the structure and function of ecosystems. While these ‘opposing’ forces have been long recognised for particular systems, debate is ongoing regarding which is more influential. Here, the influence of the bottom-up effects of nutrient availability and top-down effects of consumption (predominantly by sea urchins) are assessed on the development of macroalgae in Port Phillip Bay, southeast Australia. Field surveys across three regions showed macroalgal cover declined, and the abundance and biomass of sea urchins (*Heliocidaris erythrogramma*) increased with increasing nutrient availability. This correlative pattern was explored using a novel exclusion experiment utilising a natural barrier (the octocoral, *Erythropodium hicksoni*) that restricted access of herbivores (sea urchins and gastropods) to reef surfaces. This enabled efficient replication of plots, without grazers, in 3 regions representing different nutrient regimes. The ‘octocoral fence’ successfully excluded grazers so that after 6 months high cover of ephemeral, fast growing macroalgae had developed within grazer exclusion plots, with highest macroalgal growth occurring in the area with highest nutrient concentrations. The combined results indicate that sea urchin grazing overwhelms the positive effect of enhanced nutrients on macroalgal growth and development of macroalgal cover.

Introduction

The structure and composition of ecosystems is determined by an array of factors, many of which can be broadly classified into bottom-up (resource driven) and top-down (consumer driven) forces (Hairston et al. 1960). Spatiotemporal variability in the relative importance of these ‘drivers’ and the complex interactions between them are well recognised (Hunter & Price 1992b, Power 1992, Terradas & Penuelas 2011). The availability of resources for primary producers, such as nutrients and light, and the ‘knock-on’ effects to secondary consumers at higher trophic levels are examples of bottom-up influences. Top-down effects generally refer to consumers impacting the abundance of ‘prey’ species. Thus, both the productivity and biomass density of primary producers depends on resource availability and the magnitude of grazing pressure (e.g. Burkepale & Hay 2006). This generality holds true for shallow marine reefs able to support benthic macroalgae (Hauxwell et al. 1998, Atalah & Crowe 2010, Guarnieri et al. 2014).

Increasingly, human influences are altering the magnitude and effect of both bottom-up and top-down forces on marine ecosystems (Hiddink & ter Hofstede 2008, Noriega et al. 2012, Muthukrishnan & Fong 2014). For example, herbivore abundance can be altered through fishing either directly by removing grazers, or indirectly by removing predators of grazers, which can lead to population explosions of herbivores (Hobday et al. 2000, Tegner & Dayton 2000, Smith et al. 2001, Jenkins 2004, Connell & Irving 2008, Ling et al. 2009a). In terms of bottom-up effects, nutrification associated with human population growth in coastal areas is a focus of concern, and may include nutrient enrichment from sewage, sewage treatment facilities, and run-off, which usually increase the productivity of macroalgae (Russell et al. 2005, Elsdon & Limburg 2008, Gorman et al. 2009). It is therefore important to understand how human influences affect the balance between primary production and grazing of coastal macroalgae, so that future environmental scenarios can be identified.

The production (gross or net) potential of macroalgae can only be assessed when significant grazers are successfully excluded, which can be logistically challenging without the use of an enclosure. Excluding grazers in marine environments often requires the building of either cages (Martinetto et al. 2011) or artificial reefs (Russell & Connell 2005) and therefore these experiments are usually costly to establish and maintain, and typically require costly procedural controls to account for experimental artefacts. For this reason these kinds of experiments are often limited to only a single site and the findings are therefore

uninformative across space (e.g. Hillebrand 2003, Vanderklift & Kendrick 2005, Smith et al. 2010).

Here, octocoral colonies were utilised as an effective biological barrier to exclude herbivores, and because they were able to be efficiently replicated, the role of herbivores could be examined across different nutrient regimes. This allowed relative strengths of top-down and bottom-up effects to be assessed across shallow rocky reef systems.

Materials and methods

Regional characteristics

The work was undertaken in the urbanised embayment of Port Phillip Bay, Victoria, southeast Australia (PPB; Fig.1). PPB is Australia's largest embayment (Harris et al. 1996), and human influences on the bay include intense commercial and recreational fishing, while the surrounding cities of Melbourne and Geelong and their extensive outskirts exert ongoing influence on the Bay (Currie & Parry 1999, Fulton & Smith 2002). The study was conducted between February and August 2012 with sites representing each region situated at Long Reef (western region, S 38° 1' 35.742", E 144° 34' 58.5516"), Williamstown Beach (northern region, S 37° 52' 10.5564", E 144° 53' 36.4884") and Schnapper Point (southeastern region S 38° 12' 47.8836", E 145° 1' 56.5170") (Fig. 1). All regions exhibit extensive rocky reefs from ~ 5 m depth to the high water mark, and all reefs used in this study ranged between 3.5 and 5 m in depth.

Long Reef in the western region of PPB is close to a large sewage treatment plant (Western Treatment Plant), which services the majority of Melbourne's population (~ 4.5 M people) and causes high anthropogenic nutrient inputs into the Bay (up to 3600 t nitrogen and 930 t phosphorus annually; Harris et al. 1996). Rocky reefs in this region are dominated by extensive barrens maintained by the barrens-forming sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846; Family: Echinometridae) with densities attaining over 100 individuals m⁻² (with an average of 39 m⁻²). Macroalgae survives on individual boulders surrounded by the octocoral *Erythropodium hicksoni* (Utinomi, 1971, synonym: *Parerythropodium hicksoni*, Family: Anthothelidae) and reefs surrounded by sand (S. D. Ling pers. obs.) where hard substrates are isolated from urchin populations. The barrens habitat is predominantly devoid

of erect macroalgal growth, and is dominated by encrusting coralline algae interspersed with beds of black mussels (*Mytilus* sp.).

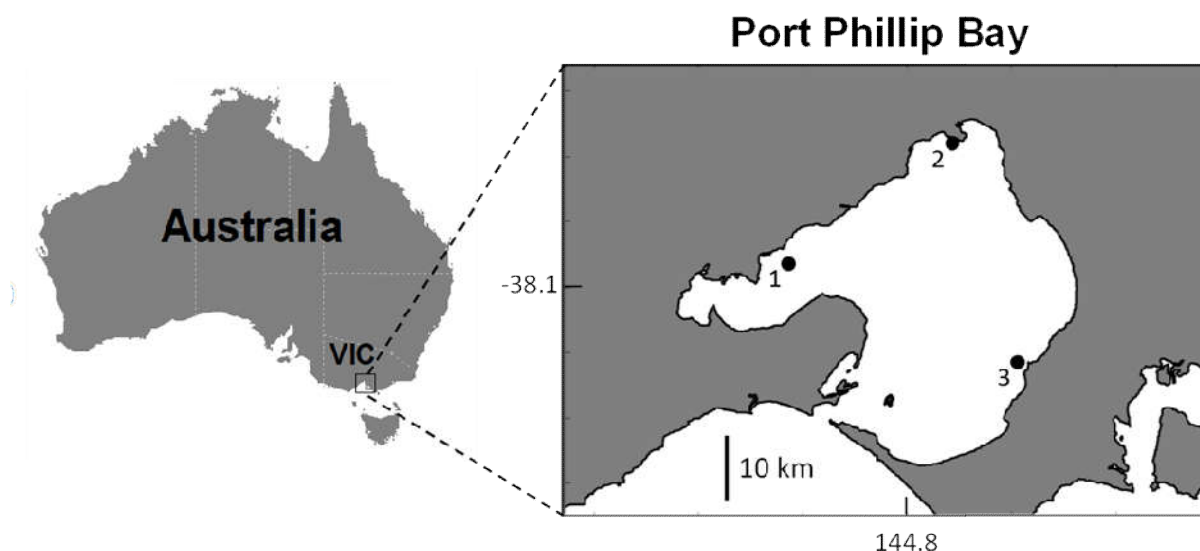


Figure 1. Map of Port Phillip Bay (PPB) Victoria, southeastern Australia; black dots in the expanded view mark the study sites in each region: 1 = west (high nutrient input), at Long Reef; 2 = north (moderate nutrient input), at Williamstown Beach; and 3 = southeast (low nutrient input), at Schnapper Point.

Reefs adjacent to Williamstown Beach in the northern region are located in the vicinity of the mouth of a major water course (Yarra River) that flows through the metropolitan area and brings up to 1700 t nitrogen and 450 t phosphorus annually into the Bay (Harris et al. 1996). The region is comprised predominantly of sea urchin barrens with small amounts of foliose algae and moderately pronounced mats of filamentous Rhodophyta and Chlorophyta, with infrequent, but dense, stands of the kelp *Ecklonia radiata* (C. Agardh) J. Agardh 1848, Family: Lessoniaceae). Mean densities of *H. erythrogramma* in this region were similar on both the urchin barrens and within the remnant kelp bed patches at 4.6 and 5.2 urchins m⁻² respectively.

Schnapper Point in the southeastern region of PPB is the most vegetated of the 3 study regions, and is located closest to the open ocean. The region is characterised by diverse kelp beds, which diminish at approximately 6 m depth where the reef transitions to urchin barrens

with infrequent occurrence of fucoids (predominantly *Sargassum* spp.), foliose Rhodophyta, and sporadic cover of turf-forming filamentous algae. Here, sea urchin densities averaged 2.4 m^{-2} in the kelp beds and 4.7 m^{-2} in the barrens habitat.

Survey methods

Percentage cover of macroalgae was assessed visually in 5% increments by a SCUBA diver using a 1 m^2 metal frame which was placed over randomly selected survey plots. Five 1 m^2 plots at each of 6 locations within each region were assessed. Urchin density was assessed concurrently with the algal cover. Density was converted to biomass density from the test diameter of $n = 10$ randomly collected urchins in each region. This was achieved by using the allometric relationship between test diameter and fresh weight determined for each region (this relationship was determined from 60 to 90 urchins sampled randomly from each region, Fig 2). Note that because of the non-linear relationship, the allometric equation was used on each urchin individually to estimate individual biomass, and then the average of these biomasses was reported.

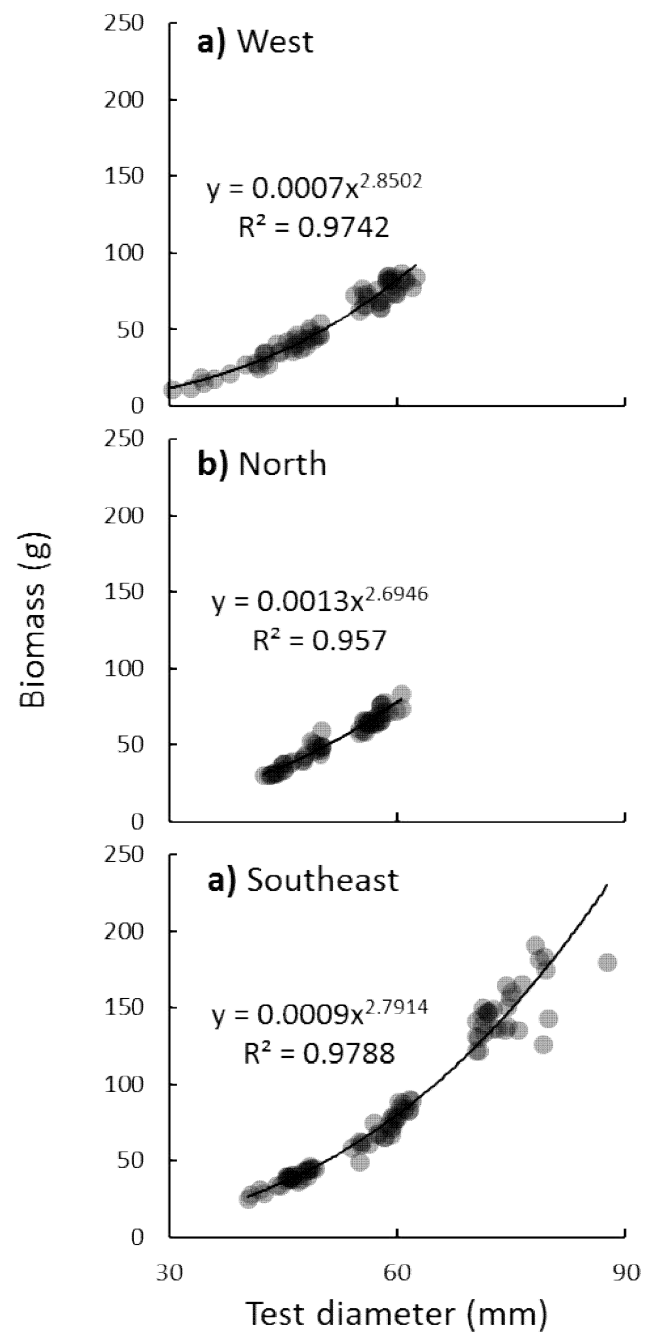


Figure 2. Allometric relationship between urchin biomass (g) and test diameter (mm) with equation and R^2 for a) the west ($n = 70$), b) the north ($n = 60$) and c) the southeast ($n = 90$) of PPB.

Water samples were taken seasonally in each region to assess nutrient levels. Three replicate seawater samples (each 60 ml) were taken at 0.3 m above the benthos. Samples were filtered, placed on ice to maintain a temperature below 5°C during transport, and then stored at -20°C (within 4 hours of collection). Samples were analysed by the Water Studies Centre, School of Chemistry, Monash University for concentrations of nitrogen (nitrogen-oxides and ammonia) and filterable reactive phosphorus (FRP).

Algal cover, urchin density, urchin biomass and nutrient concentrations were compared across the 3 regions using 1-way ANOVA. The cover of canopy-forming algae as well as all erect macroalgae differed significantly among regions, with the western region showing the lowest algal cover and the southeastern region the highest algal cover (Fig. 3a, Table 1a & b). The density and biomass of urchins also showed a significant spatial trend with highest abundances in the west and lowest in the southeast (Fig. 3b, Table 1c & d). Water samples revealed that the regions differed in regards to standing concentrations of phosphorus, but not nitrogen (Fig. 3c, Table 1e & f). Loadings of both phosphorus and nitrogen were highest in the west, followed by the north, and lowest in the southeast. Even though nutrient input into PPB is high, concentrations in the water column are low, particularly for nitrogen (Fig. 3c), reflecting high rates of denitrification. Berelson et al. (1998) reported that 63% of total nitrogen was utilised by denitrifying organisms in PPB sediments, which is high compared to most coastal areas.

Experimental design and treatments

Various means to exclude benthic herbivores were considered in a series of pilot trials, including cages, frequent culling, and construction of isolated patch-reefs surrounded by sand. Culling herbivores from patches and constructing patch-reefs were effective at low to moderate urchin densities provided they were regularly maintained (at ~ 4 weekly intervals). However, using cages was problematic as algal overgrowth caused increased shading (and in some cases collapse of cages due to intense overgrowth) within less than 2 weeks of deployment, thus demanding untenable maintenance schedules for experiments intended to be replicated over several locations and run over many months. The observed development of macroalgae within patches surrounded by the sessile colonial octocoral *E. hicksoni* even in the presence of high densities of the sea urchins, suggested octocoral as a potential useful

biological fence to sea urchins. Trials in which 200*200 mm patches on boulders were cleared of octocoral (within much larger octocoral colonies) found to successfully exclude urchins. The two other conspicuous benthic grazer groups in PPB were limpets (e.g. *Patelloida alticostata*, *Cellana tramoserica*, *Patelloida mufria*) and snails (predominantly *Australium aureum*); limpets were excluded completely from the plots surrounded by the octocoral while snails occasionally gained access to the plots.

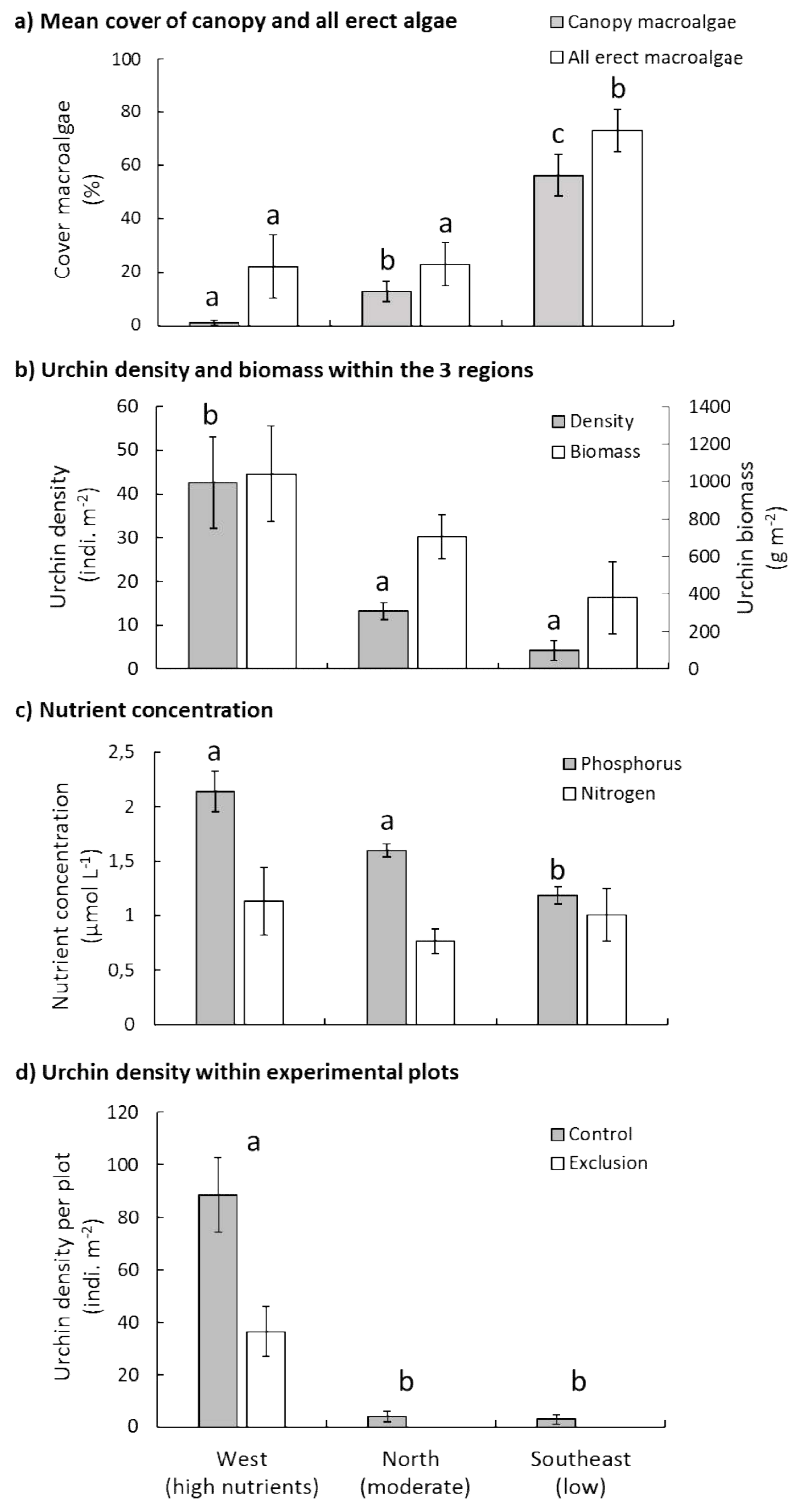


Figure 3. Description of ecological parameters and nutrient concentrations across the 3 regions of Port Phillip Bay (west, north & southeast): a) mean cover of canopy and all erect algae, b) urchin density and biomass, c) nutrient concentrations, and d) urchin densities within the experimental plots summed across the whole experiment. Data are means \pm SE. Letters within graphs indicate significant groupings (Tukey's HSD) of pairwise comparisons, $\alpha = 0.05$.

Table 1. ANOVA results for 1-way comparisons of a) background cover of all erect macroalgal, b) background cover of canopy-forming algae, c) urchin densities, d) urchin biomass density, e) phosphorus (filterable reactive phosphorus, f) nitrogen (nitrogen-oxides & ammonia) within the 3 regions of Port Phillip Bay (west, north & southeast) in 2013 and g) 2-way comparison of urchin densities within the different treatments (herbivore-exclusion & background control; fixed) and regions (west, north & southeast; fixed). Values in bold are significant at $\alpha = 0.05$.

| a) Background cover of erect macroalgae (%) | df | MS | F | p | Transformation |
|---|-----|--------|-------|---------|-----------------|
| Region | 2 | 113.65 | 7.64 | < 0.01 | $\gamma^{0.6}$ |
| Error | 15 | 14.88 | | | |
| b) Background cover of canopy-forming algae (%) | | | | | |
| Region | 2 | 25.85 | 31.39 | < 0.001 | $\gamma^{0.4}$ |
| Error | 15 | 0.82 | | | |
| c) Urchin density (m ⁻²) | | | | | |
| Region | 2 | 177.43 | 9.02 | < 0.01 | $\gamma^{0.7}$ |
| Error | 15 | 19.67 | | | |
| d) Urchin biomass (g m ⁻²) | | | | | |
| Region | 2 | 92.37 | 2.41 | 0.12 | $\gamma^{0.4}$ |
| Error | 15 | 38.3 | | | |
| e) Phosphorus (μmol L ⁻¹) | | | | | |
| Region | 2 | 0.21 | 20.43 | < 0.001 | $\gamma^{-0.5}$ |
| Error | 42 | 0.01 | | | |
| f) Nitrogen (μmol L ⁻¹) | | | | | |
| Region | 2 | 0.07 | 0.81 | 0.45 | $\gamma^{-0.5}$ |
| Error | 27 | 0.09 | | | |
| g) Urchin density within exclusion and control plots (m ⁻²) | | | | | |
| Treatment | 1 | 57.5 | 10.1 | < 0.01 | $\gamma^{0.9}$ |
| Region | 2 | 387.6 | 67.96 | < 0.001 | |
| Treatment x Region | 2 | 2.3 | 0.41 | 0.67 | |
| Error | 138 | 0.05 | | | |

In this experiment, focus was on the exclusion of sea urchins as they represented the most active grazer with the highest biomass density. Herbivorous fish (e.g. *Parma victoriae*, *Girella zebra*) were not considered in this experiment because they occurred rarely at the sites and other controlled trials showed negligible effects of their grazing on local macroalgae. To assess grazing by herbivorous fish, freshly cut algal pieces (*E. radiata*, 50 x 20 mm lateral fronds, $n = 48$ in total in 4 regions of PPB) were attached to fishing line and a small float, so that they hovered within the algae canopy. The trial was conducted in all regions of PPB and showed zero loss of biomass of algae pieces over 3 days. No statistical analysis was conducted as no grazing of algae pieces occurred ($0.0 \pm 0.0 \text{ mm}^2$). Given undetectable grazing by herbivorous fishes and successful exclusions of benthic herbivores, the 'octocoral urchin-exclusion plots' were considered to be effectively herbivore-free. In addition, edge-effects were undetectable given the low relief of the octocoral colonies and regenerated algae within the octocoral clearance plots grew to the boundary defined by the presence of the octocoral. The only required maintenance of the octocoral exclusion plots was periodic pruning of the octocoral edges every 2 to 3 weeks to ensure that the colony did not grow back into the cleared area. The average growth rate of *E. hicksoni* edges was 3 mm ($\pm 0.5 \text{ mm SE}$) per 2.5 weeks (on average) between maintenance events. Given growth of algae to the edge of the octocoral and the fact that no other kind of barrier was used to prevent urchins from entering, no procedural controls were required.

Within each region, reef sites dominated by urchin barrens with a high abundance of the octocoral *E. hicksoni* were selected, and rocks > 600 mm diameter completely covered by octocoral colonies were selected as experimental units. Eight large colonies of *E. hicksoni* were chosen in each region, each situated on the flat top of large boulders with less than 45° slope relative to the vertical. Plots (200*200 mm) were cut from the centre of colonies, ensuring that the removal areas remained surrounded by at least 100 mm of octocoral on all sides. All underlying organisms were removed and the rock surface was scraped clean. After preparing each plot, a 200*200 mm 'control' patch was randomly chosen on a nearby boulder with no octocorals, which was marked by a stainless steel pin secured to the benthos with Z-Spar epoxy. The 'control' patch was left untouched so that the naturally occurring algal community could be monitored. In this way the development of algae could be compared to the background cover of algae accessible to benthic grazers (Fig. 4). Treatments were classified by 'region' (3 levels; west, north and southeast) and 'herbivory' (2 levels;

exclusion and background control), with $n = 8$ replicates of each treatment and region (given a total of 48 experimental plots).



Figure 4. Photo of the setup of the experiment. The photo shows the exclusion plot at the bottom and the control plot at the top. Around the control plots the metal frame can be seen, which was placed around both plot types during assessments and maintenance visits to ensure the correct area was assessed, and to quantify octocoral regrowth into the cleared area.

Assessments of macroalgal development were conducted after 2 months of exclusion to examine any response to the newly available space, and repeated after 6 months. The experiment was then terminated after ~ 7 months because some octocoral colonies were changing their growth form and beginning to break down along at least one edge allowing ingress of herbivores. Planar percentage cover of all macroalgal species within each plot, plus abundances of sea urchins, were determined *in situ* on SCUBA by using a 200*200 mm metal frame which was placed around the plot to then estimate % cover visually. Maintenance was

required every 2 to 3 weeks to trim the octocoral growth and to ensure plots remained free from herbivores. Urchin abundance was successfully reduced in the exclusion plots in the west (Fig. 3d) while urchins were excluded completely in the north and southeast (Table 1g). The ingress of urchins into experimental plots in the western region was likely due to recruitment and rapid growth of the introduced annual kelp *U. pinnatifida*, which enabled urchins to move into the exclusion plots by climbing onto the sporophyte laminae when they lay outside the plots, providing a bridge across the octocoral surface.

Data analysis

Univariate responses were analysed using R (The R Foundation for Statistical Computing, Version 2.15.1, © 2012). Differences in mean algal cover among treatments after 6 months were evaluated using a 2-way ANOVA, with ‘treatment’ (2 levels: control and exclusion) and ‘region’ (3 levels: west, north and southeast) as fixed factors, and cover of fleshy algae (i.e. excluding turf algae) as response variables. Where necessary, transformations to stabilise variances were determined by the Box-Cox procedure in R (available in the MASS package). To test the multivariate ‘community response’ of all algal species simultaneously (% cover), a Permutational Multivariate Analysis of Variances (PERMANOVA) was conducted using the PRIMER software (Version 6.1.12 & PERMANOVA+ Version 1.02, © 2009 PRIMER-E Ltd.). For univariate analysis, the factorial design consisted of the 2 factors, treatment (control and exclusion) and region (west, north and southeast). A similarity analysis using SIMPER was conducted to identify the algae contributing to the differences between groups. To visualise the multivariate analysis a canonical analysis of principal coordinates was used to project an overview of the structure of the data and the separation of the factors.

Results

Macroalgal cover response

After 2 months of herbivore exclusion, clear regional patterns of macroalgal growth were observed. The western region showed the strongest immediate response of macroalgal growth, followed by the northern and then southeastern region. Relative to the other two

regions, in the southeast there was less algal growth in exclusion plots than in the background control plots (Fig. 5).

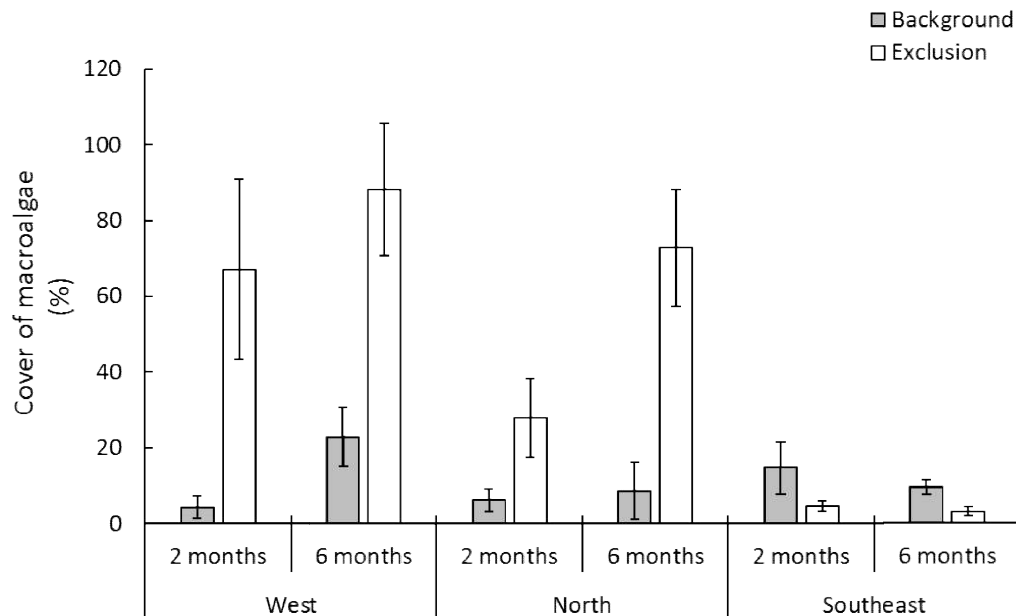


Figure 5. Percentage cover of macroalgae at 2 months and 6 months after exclusion of benthic herbivores (essentially sea urchins). Mean macroalgal cover is shown for urchin exclusion plots (white bars) and ‘control’ plots to which urchins have access (grey bars) \pm SE. Statistical analysis was only conducted on the data after 6 months with a significant ‘treatment’ and ‘region’ interaction ($F_{2,42} = 10.98$, $p < 0.001$). Asterisks indicate a significant difference between exclusion and control plots (Tukey’s HSD).

After 6 months of exclusion, a 2-way ANOVA revealed a significant ‘treatment’ effect ($F_{2,42} = 12.68$, $p < 0.001$, transformation = $Y^{0.25}$), site ($F_{2,42} = 8.57$, $p < 0.001$), and ‘treatment by region’ interaction ($F_{2,42} = 10.98$, $p < 0.001$). Tukey’s post-hoc HSD showed that herbivore-exclusion in the western and northern regions led to significant increase in macroalgal cover compared to control plots, while in the southeast, macroalgal cover in the exclusion plots did not differ significantly to that in the control plots and had not reached the cover of the control plots.

Macroalgal community response

Comparison of algal communities between herbivore-exclusion and background control plots after 6 months also identified a significant ‘region by treatment’ effect (PERMANOVA, transformation = square root, distance measure = Bray-Curtis, $F_{2,42} = 3.67$, $p = 0.001$). Pairwise comparison showed differences in algal community composition in exclusion and background control plots in the north and in the southeast, but not in the west ($t = 2.77$, $p = 0.001$; $t = 2.1$, $p = 0.003$; $t = 1.57$, $p = 0.08$ respectively; Fig. 6). In the north the algal cover of the control plots was dominated by *Colpomenia* sp. (93%), whereas *Ulva* sp. accounted for the highest cover (95%) in the exclusion plots. In the southeast, dominant algal species were *Dictyota dichotoma* ((Hudsun) J.V. Lamouroux 1809, Family: Dictyotaceae) in the control plots and *Sargassum* spp. and *Ulva* sp. when herbivores were excluded (48%, 36% and 35% respectively). The species contributing most to the similarity in algal community structure in the west between the exclusion and control plots were *Ulva* sp. and *Undaria pinnatifida* ((Harvey) Suringar, 1873, Family: Alariaceae), which together almost exclusively dominated reefs in this region. This dominance was apparent for both exclusion and control plots, but in the exclusion plots mean cover of *Ulva* sp. was higher than that of *U. pinnatifida* (61% and 39% contribution), whereas the opposite pattern was evident in the control plots (20% and 73% for *Ulva* and *U. pinnatifida* respectively).

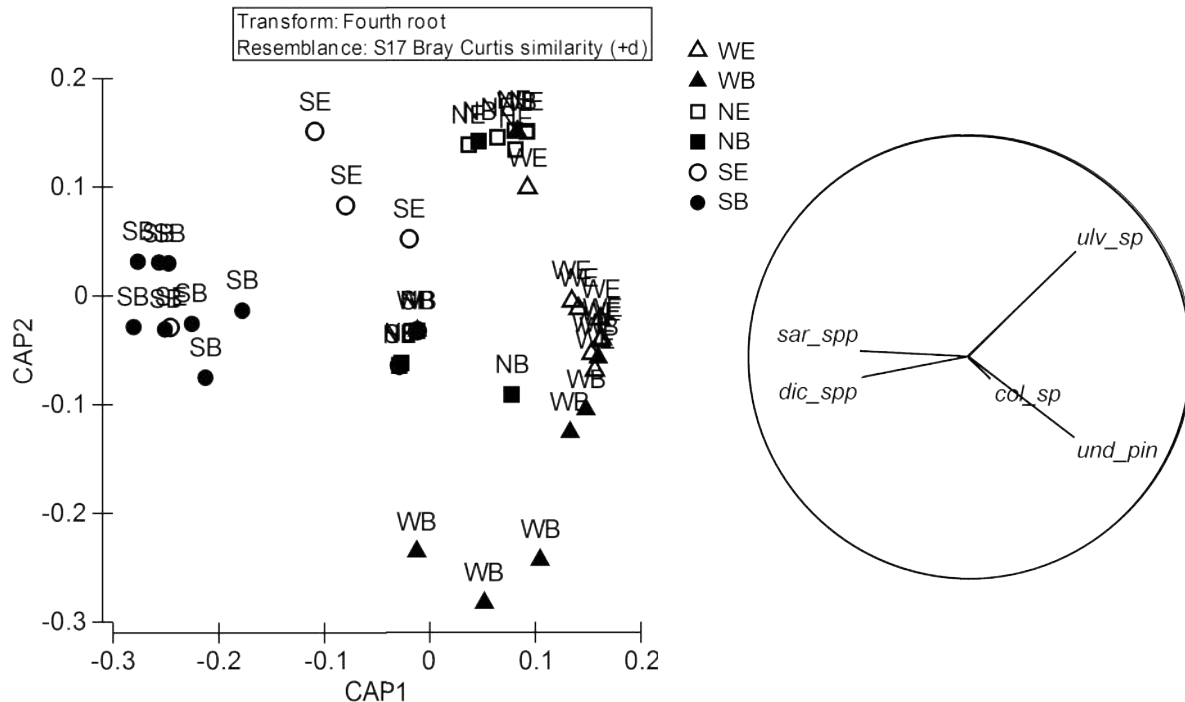


Figure 6. Canonical analysis of principal coordinates ordination plot based on algal community composition for each combination of treatment (E = herbivore-exclusion plots, open symbols; B = ‘control’ plots, filled symbols) and region (W = west, N = north, S = southeast) after 6 months. Circle identifies vectors of algae species contributing most to differentiation of groups on the ordination plot (*sar_spp* = *Sargassum* spp., *dic_spp* = *Dictyota dichotoma*, *und_pin* = *Undaria pinnatifida*, *col_sp* = *Colpomenia* sp. and *ulv_sp* = *Ulva* sp).

Discussion

Previous studies have shown that algal communities differ in response to variations in water quality or ‘bottom-up’ forces (Duarte 1995, Eriksson et al. 2002), however rarely have algal communities been examined across nutrient regimes in concert with an experiment controlling ‘top-down’ effects. Using octocoral as a natural biological barrier, grazers were successfully excluded across several regions of differing nutrient regimes to provide insight into the correlative relationships between these opposing forces. The rarity of investigations examining the relationship between algal abundance, community composition, levels of nutrient and herbivory across multiple regions arguably reflects the difficulty of

simultaneously running manipulative field experiments with suitable levels of replication across multiple regions.

Effectiveness and efficiency of a natural biological fence

The ability to effectively manipulate the sub-tidal reef system of PPB was made possible by the observation of macroalgae growing in small (sub-metre scale) patches within colonies of the octocoral *E. hicksoni*, demonstrating that the octocoral provided a natural barrier to movement of sea urchins. The widespread availability of the octocoral on the reefscape avoided the need to use and maintain foreign or artificial surfaces (e.g. settlement tiles; see Hills & Thomason 1998) and structures (e.g. cages or fences) as barriers to urchin movement, obviating the requirement for frequent maintenance (reviewed by Miller & Gaylord 2007) and elaborate procedural controls. Similarly, mesocosms have the same disadvantages as cages, but face the additional problem of being an even more artificial environment and do not accurately reflect natural habitats (Lalli 1990, Kraufvelin 1999).

In the present experiment, sheets of octocoral (*E. hicksoni*) were used to provide natural barriers to urchin movement. This technique allowed for rapid set-up times and required minimal maintenance. The only maintenance required was regular trimming of the octocoral colony around the edges of the removal area, which was easily accomplished. An edge effect was considered to be minimal as algae were observed growing in the direct vicinity of the octocoral sheets. The disadvantage of natural barriers of this type is that, as living organisms, change in growth patterns and compromise of the barrier along any edge of the colony is a potential risk. This occurred in plots in the southeast after 6 months, at which time the experiment was terminated. A potential limitation of the method is the size of the experimental units. Due to the size of the octocoral colonies, the experimental units could not be greater than 20 x 20 cm, constraining the scale of the experiment. Nonetheless, this size was sufficient to assess colonisation of surfaces and settlement / development of organisms, and enabled replication across space which is rare for herbivore exclusion experiments that are almost always performed at a single site (and then generalised across space by inference). Furthermore, the use of settlement surfaces, most commonly tiles, are routinely used in research (e.g. Smith et al. 2001, Worm & Lotze 2006, Albert et al. 2008, Fabricius et al. 2015) and most of the experimental units used are smaller than the 400 cm² used here (typically between 50 and 150 cm²).

Macroalgal cover response

The most rapid response in macroalgal cover to exclusion of herbivores was in the west of PPB, in the most urbanised region with heaviest anthropogenic nutrient input. The northern region, with intermediate nutrient input ranked second in terms of gross macroalgal response independent of herbivory. In the southeast very little development of macroalgal cover in the exclusion plots was found and after 6 months the cover in these plots remained less than the cover in the background. This region is closest to the open ocean and exhibits the lowest nutrient concentrations and least impact of urban development, but the highest standing cover of perennial macroalgae (Fig. 3b). The control plots in this study were treated differently than the grazer exclusion plots since the controls were not scraped clean. Thus the community that developed in the exclusion plots were from initial colonisers whereas the communities observed in the control plots were long established. It is striking however, that in a relatively short time the cover of macroalgae in the grazer exclusion plots that were initially cleared more than tripled compared to the natural occurring cover in background control plots in the 2 higher nutrient regimes. This initial bloom in macroalgae indicates the importance of the top-down influence within this system.

The exclusion of herbivores across sites of different nutrient regimes demonstrates that in the absence of grazers, the rate of gross accumulation of algal cover is consistent with nutrient availability. This is in accordance with other studies, in which colonisation of algae increases when nutrient levels were enhanced artificially (Korpinen et al. 2007, Guarnieri et al. 2014). For example, Guarnieri et al. (2014) found that recovery of erect algae would only occur when grazers were excluded, but was significantly higher in plots with added nutrients. Similarly, several studies reported increased algal growth, particularly of fast-growing species, when nutrients were enhanced (Duarte 1995, Bokn et al. 2003). This notion correlates with the algal communities found in the west and north of PPB that are dominated by fast growing opportunistic ephemeral species, such as *Ulva* sp and *Codium* spp., in contrast to the southeast, where *Sargassum* spp. was the dominant species which grows relatively slowly but is perennially present. Interestingly, there was no evidence that filamentous turf-forming species proliferated at the expense of foliose or fleshy macroalgae in areas with greatest loading of anthropogenic nutrients, as has been observed in other studies (Gorgula & Connell 2004, Russell & Connell 2005, Russell et al. 2009). These studies from South Australia found that newly available space was occupied by turf-forming algae,

particular when grazers were absent. In PPB, even though turf communities are present (especially in the west and north) the algae that developed within our experimental plots were foliose algae.

Macroalgal community response

In the north and the southeast, community structure differed between exclusion and control plots. In the north the algal community in the control plots showed sparse cover dominated by *Colpomenia* sp., whereas the exclusion plots supported a dense cover of *Ulva* sp. Typically, high nutrient inputs stimulate the growth of foliose species like *Ulva* sp. in the absence of grazers (Lobban & Harrison 1994), but the high palatability of this species makes it susceptible to overgrazing by urchins, particularly since *Ulva* sp. is a preferred food of urchins (Cyrus et al. 2015). In the southeast, cover of algae in exclusion plots after 6 months was minimal, and did not attain background levels of the area, confirming that the region has relatively low productivity, contrasting the other regions examined. Yet while the rate of macroalgal recovery was very slow in this region, the macroalgal diversity was highest (Fig. 3a). Indicating that, in concordance with other studies (Worm et al. 1999, Gorgula & Connell 2004), more diverse algal communities tend to develop in low nutrient regimes.

In the west, the dominant macroalgae were *Ulva* sp. and *Undaria pinnatifida*, with *U. pinnatifida* showing higher cover in control plots and *Ulva* sp. in exclusion plots. This suggests that either *U. pinnatifida* is less palatable to *H. erythrogramma* than *Ulva* sp., or and more likely, that at the time during which the experiment was conducted there was no recruitment of *U. pinnatifida* sporophytes (which is highly seasonal). *U. pinnatifida* originates in the Northwest Pacific and was introduced to PPB at the end of the 1990s (Campbell & Burridge 1998). It is a fast-growing annual canopy-forming species, which builds dense cover in western PPB, whereas it occurs frequently, but is not as abundant in the north and is observed infrequently in the southeast. Valentine and Johnson (2005b) showed that *H. erythrogramma* readily consumes *U. pinnatifida* in Tasmania, and similarly it was consumed by urchins in PPB, however the urchins' preference rankings for different algae species have not been determined.

Conclusions

Using colonies of octocoral can be a cost and time-effective way to conduct small-scale grazer exclusion experiments with high numbers of replicates. This technique additionally enables experiments to be repeated at different locations and in this way allow insight into how the algal community changes with different environmental conditions. These qualities make it a valuable tool for studying the dynamics of diverse marine ecosystems such as rocky reefs. In this study, it was found that the southeast region, which supported the greatest alpha diversity of macroalgae and greatest area of intact kelp forest within PPB, showed the lowest algal growth in response to grazer removal consistent with low nutrient availability in this region. Interestingly, urchin density was positively correlated with nutrient availability, showing highest densities in the west and lowest in the southeast, so that the (top-down) capacity for destructive overgrazing by urchins is higher where nutrient availability (bottom-down) is greater. Due to this characteristic, effects of urchin grazing overwhelm the stimulation of algal growth by higher nutrient availability.

A potential reason for the high abundance of urchins in the west could be that higher nutrient availability and therefore algal productivity sustains higher herbivore abundance. Unfortunately, studies in which urchin abundance is linked to environmental factors, such as nutrient availability within a system are rare and therefore no conclusion can be made about the generality of this observation. However, if this pattern is observed on other temperate reefs globally, i.e. that the top-down control of sea urchins increases with nutrification, it would suggest that the bottom-up effect of nutrients could indirectly strengthen the top-down effect of grazing. Nutrient enhancement could be a useful tool in low nutrient areas to accelerate recovery of macroalgae, but only once grazers have been removed from the system (Korpinen et al. 2007, Guarnieri et al. 2014). However, such nutrification can potentially lead to a stimulation of the growth of opportunistic, rapidly growing algae such as turf forming algae (Gorgula & Connell 2004, Russell & Connell 2005, Atalah & Crowe 2010). This often leads to a formation of a semi-consolidated matrix of turf alga and sediment that inhibits the recruitment of kelp and other canopy-forming algae (Valentine & Johnson 2005a, Irving et al. 2009). It is apparent that even though the top-down force of grazing by urchins drives the abundance of macroalgae in PPB, the bottom-up force of nutrients plays an important role in the composition of the algal community by ameliorating the growth of fast-growing annual species. With the ongoing urbanisation and thus nutrification of coastal areas there is a high

risk of a transition of rocky reef systems to dominance by opportunistic and undesirable macroalgal species.

Chapter 3: Top-down sea urchin overgrazing overwhelms bottom-up nutrient stimulation of urban kelp beds

Abstract

Biogenic marine habitats are increasingly threatened by a multitude of human impacts, and temperate coasts in particular are exposed to progressively more intense and frequent anthropogenic stressors. In this study, the single and multiple effects of the urban stressors of nutrification and sedimentation on kelp bed communities were examined within Australia's largest urbanised embayment (Port Phillip Bay, Victoria). Within this system, grazing by sea urchins (*Heliocidaris erythrogramma*) plays an important role in structuring reef communities by overgrazing kelp beds and maintaining an alternative and stable urchin barrens state. It is therefore important to explore the effects of urban stressors on kelp bed dynamics related to urchin abundance, and test the relative strengths of bottom-up and / or physical drivers (e.g. elevated nutrients and sediment) *versus* top-down (e.g. urchin grazing) forces on kelp bed community structure. The interactions of these drivers were assessed to determine whether their combination has synergistic, antagonistic, or additive effects on kelp beds. It was found that kelp responds positively to nutrient enhancement, but when combined with enhanced abundance of grazing sea urchins, the local positive effect of nutrient enhancement is overwhelmed by the negative effect of increased herbivory. Turf-forming algae behaved very differently, showing no detectable response to nutrification, yet showing a positive response to urchins, apparently mediated by overgrazing of canopy-forming algae that limit turf development. No direct effects of enhanced sediment load (at twice the ambient load) were found on intact kelp beds. Collectively, the results demonstrate that the 'top-down' control of urchin grazing locally overwhelms the positive 'bottom-up' effect of nutrient enhancement, and that intact kelp beds demonstrate resilience to direct impacts of urban stressors.

Introduction

Ecosystems are shaped by a complex arrangement of factors that have historically been conceptualised by multiple ecological paradigms that are often presented, explicitly or implicitly, as mutually exclusive. Contrasting conceptualisations include ecological processes driving ecosystem structure that emanate from either high or basal trophic levels, i.e. as either ‘top-down’ or ‘bottom-up’ control (Hairston et al. 1960, Hunter & Price 1992a, Worm et al. 1999). Top-down refers to consumer control that can cascade down through successive trophic links in a food-chain, while bottom-up forces flow through the food-web from the primary production base, influenced by resource-driven forces such as availability of nutrients and light. Determining where and when these different ‘forces’ shape marine ecosystem structure has received considerable focus (reviewed by Menge 2000), but there are few experimental studies that have simultaneously considered their dual roles within a particular system (e.g. Smith et al. 2010, Sjöo et al. 2011, Vinuela et al. 2014).

Worldwide, an increasing intensity and frequency of human-derived stressors is poised to change the relative strength of top-down *versus* bottom-up control mechanisms in natural ecosystems. For marine environments, anthropogenic impacts of climate change (Hiddink & ter Hofstede 2008, Ling et al. 2009b, Last et al. 2011), pollution (Mearns et al. 2011), and the urbanisation of coastal areas (Noriega et al. 2012) can be directly linked to substantial changes in the configuration of marine communities and ultimately to the goods and services they provide (reviewed by Strain et al. 2014). Examples documenting the impacts of individual stressors are accumulating, but ecosystems are innately complex and are typically subjected to multiple stressors simultaneously, particularly as anthropogenic influences augment the intensity and frequency of stressors (Muthukrishnan & Fong 2014). When several stressors interact, three qualitative outcomes are possible; the impact of multiple stressors can be: (1) additive, equal to the sum of their individual effects; (2) antagonistic, so that the response is less than the sum of their separate impacts or (3) synergistic, when the impact is greater than the sum of the individual effects. Consequently, the effects of multiple stressors can vary in space and time and create complex and indirect ‘downstream’ effects (Paine et al. 1998) that can be difficult to predict. To support informed management of a particular system, it is necessary to understand the effects of multiple anthropogenic stressors, be they related to climate change, harvesting, or urbanisation (Ling et al. 2009a, Boyd & Hutchins 2012, Annala et al. 2014).

For algal communities on urbanised coasts, three primary stressors of global concern are enhanced sedimentation, increasing nutrient loads (as bottom-up forces), and altered herbivory (a top-down force) (Airoldi 2003, Connell 2005, Russell & Connell 2005, Strain et al. 2014). Sediment and nutrient loading in many coastal systems are increasing due to anthropogenic-driven changes to catchment and run-off processes, and can result in a shift to a dominance of low-lying algal turfs over more structurally and species diverse kelp beds (Airoldi 2003, Strain et al. 2014). Altered grazing pressure is frequently driven by the cascading impacts of overfishing of the herbivores leading to declining population density (Smith et al. 2001, Connell & Irving 2008) or overfishing of their predators leading to increased population density (Tegner & Dayton 2000, Ling et al. 2009a). While there are several studies on the direct effect of these stressors acting in their own right (Pfister & Van Alstyne 2003, Karez et al. 2004, Eriksson & Johansson 2005, Balata et al. 2007), and research considering two stressors simultaneously and the nature of their interactive effects is gaining momentum (e.g. Gorgula & Connell 2004, reviewed by Strain et al. 2014), very few studies have considered more than two stressors in combination in *in situ* field experiments in marine subtidal habitats (for rocky shores see Atalah & Crowe 2010).

In the present work, the individual and combined effects of three potential stressors on kelp bed dynamics were assessed in a 14 month field experiment in Australia's largest embayment, Port Phillip Bay (PPB). Nutrient enrichment, sediment enhancement, and sea urchin herbivory were manipulated to test for their effects singly and in factorial combination on the community structure of shallow rocky reefs in the Bay.

Materials and methods

Study area

Port Phillip Bay lies adjacent to Australia's second largest metropolitan area (Melbourne, with ~ 4 million people), and is one of Australia's largest embayments (with an area of 1,930 km² and a water volume of 26 km³ (Harris & Crossland 1999). It has been subject to a range of anthropogenic impacts since European settlement, and from the early 19th century anecdotal evidence suggests widespread decline of kelp bed habitats and heavy impacts of fishing on predators such as spiny lobsters (Jung et al. 2011). Spiny lobsters are important

predators of sea urchins in the south-eastern Australian region (Pederson & Johnson 2006, Ling et al. 2009a).

This study was conducted within a kelp bed at Schnapper Point, on the Mornington Peninsula (south-eastern PPB, S 38° 12' 47.8836", E 145° 1' 56.517") during the 14 month period December 2012 to February 2014. Sub-tidal rocky reefs at this site are characterised by large boulders (up to ~ 3 m diameter) and cobbles (to ~ 100 mm diameter) down to the sand-edge at 7 m depth. Sea urchin barrens and kelp habitat co-occur throughout the area with kelp beds dominating shallower areas and barrens habitat occurring at depths of 5 to 7 m. Kelp bed habitat consists of a variety of canopy-forming algae (primarily *Ecklonia radiata*, *Sargassum vestitum*, *Sargassum decipiens*, *Sargassum fallax*, *Cystophora moniliformis*, and *Cystophora monilifera*), a broad range of fleshy understorey algae (mainly *Zonaria* spp., *Lenormandia marginata* and *Exallosorus olsenii*) and turf-forming algae. Turf algae were defined as filamentous algae (typically < 40 mm in height) which form a semi-consolidated matrix with trapped sediments, but were not identified to species level. The barrens habitat shows a sparse presence of *Sargassum vestitum* and marginal establishment of turf-forming algae. The most abundant macro-herbivore is the sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846; Family: Echinometridae) with average density of 5.7 m⁻² on urchin barrens and 2.4 m⁻² in kelp bed habitat (biomass: 746 g m⁻² and 500 g m⁻² respectively).

Experimental design and data collection

Within the Schnapper Point kelp bed, 24 1 x 1 m plots at a depth of ~ 4 m were marked with 2 star pickets, 1 at the corner and 1 in the centre. Initial surveys of experimental plots prior to manipulation showed an average of 53% cover of canopy-forming species ($\pm 1.9\%$ SE, including 39% $\pm 1.5\%$ SE cover of *E. radiata*), 84% cover of understorey ($\pm 4.5\%$ SE), and 66% cover of turf-forming algae ($\pm 4.0\%$ SE). The average sediment depth within the turf community was 2.7 mm (± 0.2 mm SE). The field experiment consisted of factorial combinations of 3 treatments each with 2 levels (enhanced and ambient) and 3 replicates applied randomly to the 24 1 x 1 m plots. The treatments were as follows:

Nutrient enhancement: Mesh bags (1 mm mesh size) were cable tied to the middle star pickets and either filled with 200 g of Osmocote® Pro (3-4 M, 17N:4.8P:8.3K) for enhanced nutrient conditions or with pebbles as procedural control for ambient nutrient conditions. To

ensure a constant supply of elevated nutrients the bags were replaced every 6 weeks. This method of applying fertiliser and the calculation of the nutrient dosage were in accordance with Worm et al. (2000). To ensure independency of experimental units (see Worm et al. (2000) and Russell and Connell (2005)), each plot was at least 5 m away from its nearest neighbour. The effect of nutrient enhancement, and also the adequacy of the 5 m buffer between nutrient treatment and control plots, was confirmed by analysing nutrient levels in water samples taken half way through one fertiliser application (3 weeks after exchange of mesh bags). Water samples were taken with a syringe at 200 mm from the mesh bags just above the highest point within the plots, then filtered into 60 ml falcon tubes and kept on ice for approximately 2 hours during transport before being frozen. The samples were tested for concentrations of nitrite, nitrate, ammonia and filterable reactive phosphorus at the Water Studies Centre, School of Chemistry, Monash University, Victoria. Results were analysed with 1-way ANOVAs with ‘nutrient treatment’ as a factor (2 levels: ‘ambient’ and ‘enhanced’) and concentrations of ammonia, nitrogen-oxides, filterable reactive phosphorus and all nutrients combined as response variables. The analysis showed significantly higher concentrations of nitrogen-dioxides and total nutrients at sites with added Osmocote than at the control sites, while differences in ammonia and phosphorus were not significant due to high variability in measurements within ‘treatments’ (Table 1, Fig 1a).

Table 1. Mean values and results of 1-way ANOVAs of nutrient concentrations for ‘Ambient’ and ‘Enhanced’ nutrient conditions on experimental plots. Mean values ($\mu\text{mol l}^{-1} \pm \text{SE}$, $n = 6$) and results of ANOVAs are shown for ammonia (NH_3), nitrate and nitrite combined (NO_x), filterable reactive phosphorus (FRP) and the sum of all nutrients (Total). Values in bold are significant at $\alpha = 0.05$. The transformations used were $\ln(Y)$ for NH_3 , Y^1 for NO_x and FRP, and $Y^{1.5}$ for Total nutrients.

| | Ambient ($\mu\text{mol l}^{-1}$) | Enhanced ($\mu\text{mol l}^{-1}$) | <i>df</i> | <i>MS</i> | <i>F</i> | <i>p</i> |
|-----------------------|---------------------------------------|--|-----------|-----------|----------|-------------------|
| NH3 | 0.43 (± 0.12) | 1.63 (± 0.67) | 1 | 0.63 | 3.64 | 0.09 |
| NO_x | 0.04 (± 0.003) | 0.27 (± 0.11) | 1 | 1486.70 | 69.08 | < 0.001 |
| FRP | 1.39 (± 0.11) | 1.86 (± 0.23) | 1 | 0.09 | 4.23 | 0.07 |
| Total | 1.85 (± 0.14) | 2.41 (± 0.11) | 1 | 0.14 | 13.19 | < 0.01 |

Sediment enhancement: Every 6 weeks the equivalent of ~ 6 weeks of sediment deposition occurring at the study site was added to the experimental plots by divers *in situ* (18.1 kg m⁻² dry weight, Fig. 1b), i.e. this treatment doubled the ambient sediment load. Ambient sediment load was calculated from sediment deposition rates measured by using sediment traps deployed at the study site for 365 days prior to the start of experiments (Sediment traps consisted of PVC piping 50 mm in radius and 500 mm in length, capped at the bottom and meshed with 10 mm grid baffles at the top). Traps were deployed and retrieved using SCUBA every 6 to 8 weeks and the sediments were air dried to constant weight at 60°C. The dry weight of the samples provided an estimate of mean deposition rate of 430.5 g m⁻² d⁻¹. This rate is consistent with values presented by Connell (2005) for urban coasts in South Australia and the sediment enhancement treatment occurred within the range of sedimentation rates documented by Connell (2005) for harbour areas (Fig 1b), which were considered stressful for kelp beds.

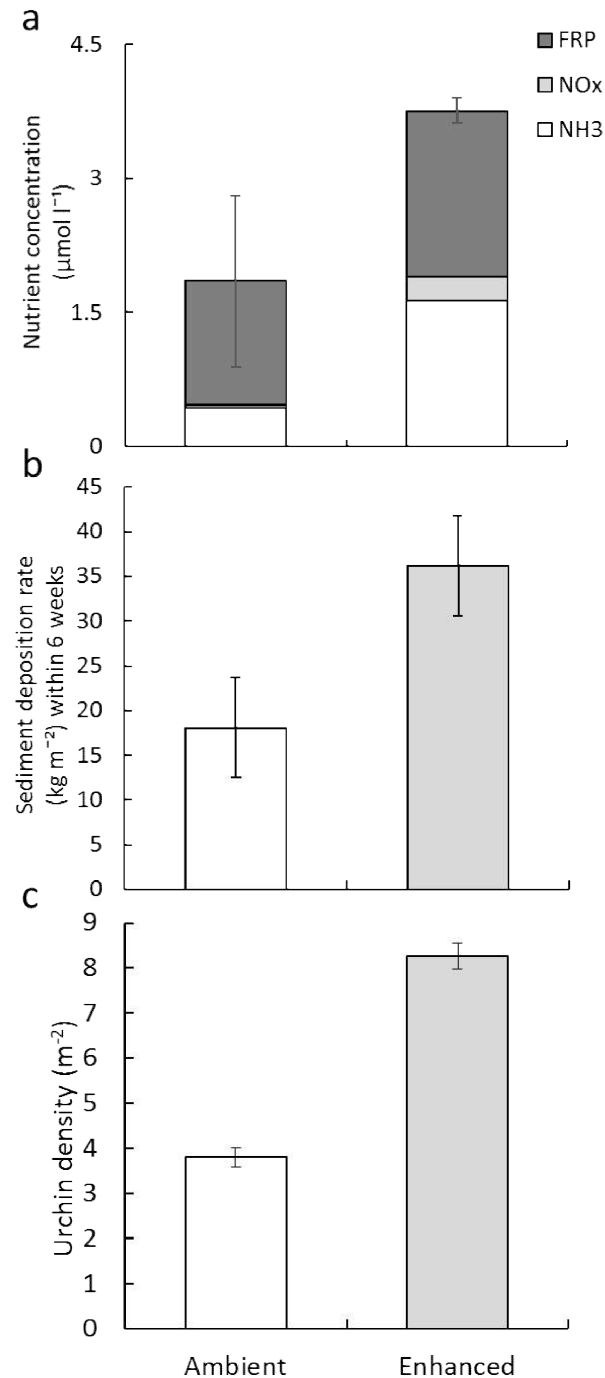


Figure 1. Overview of treatment magnitudes applied throughout the 14 months of the experiment. a) Nutrient concentration of ambient and enhanced nutrient plots 3 weeks after applying nutrient treatment (means \pm SE of all nutrients combined); FRP = filterable reactive phosphorus, NOx = nitrogen-oxides and NH3 = ammonia. b) Amount of sediment naturally deposited on the reef (white bars) and amount of natural plus experimental sediment deposited every 6 weeks (grey bar, means \pm SE). c) Sea urchin densities (mean no. individual $\text{m}^{-2} \pm$ SE) in plots with ambient and enhanced urchin abundance time-averaged over 8 maintenance events during the entire 14 months of the experiment.

Sediment used for the enhancement treatment was sourced by divers using a hand scoop and screw-top barrel from a nearby sub-tidal sand flat, which matched grain size distribution as observed in the local sediment traps (mainly fine sand $\sim 200\ \mu\text{m}$ and medium sand $500 - 700\ \mu\text{m}$). These grain sizes were chosen (1) because they reflected the composition of the naturally occurring sediment deposition, and (2) because it would act as a stressor in two ways, with the smaller particles having the potential to smother microscopic algal stages while larger particles could act as a stressor by causing abrasion. Thus these different potential mechanisms of sediments as stressors on the algal community could be assessed. The collected sediments were poured into a calibrated 60 L header tank on the research vessel and then gravity fed, in combination with centrifugal force generated by stirring, to the experimental plots via a 6 m long, 30 mm diameter clear plastic hose. The hose was handled *in situ* by a SCUBA diver who ensured the standardised sediment load was evenly spread over treatment plots. Sediment additions were conducted on days with low swell and calm weather when the weather forecast predicted a calm period to come.

Urchin enhancement: Every 4 to 6 weeks sea urchins were added to treatment plots by divers to maintain a minimum density of $8\ \text{m}^{-2}$. At each maintenance event, densities were assessed in plots receiving both the ‘ambient’ and the ‘enhanced’ treatment before the number of sea urchins in enhancement plots was topped up, to examine whether the urchin enhancement treatment was successful. Urchin densities were significantly greater in enhanced plots (average urchin density of $8.3 \pm 0.3\ \text{m}^{-2}$) than in ambient plots (average urchin density of $3.8 \pm 0.2\ \text{m}^{-2}$) over the duration of the 14 month experiment (Dec 2012 to Feb 2014, minimum p -value = 0.0001; see Fig. 1c). Sea urchins were collected from the same kelp bed at a minimum distance of at least 10 m from experimental plots, and were placed in the middle of the plots within crevices to avoid rapid emigration.

Assessment of the percentage cover of macroalgal species and of algal turf within the plots was determined every 3 months. Percentage cover was assessed in 5% increments by a SCUBA diver *in situ* using a $1\ \text{m}^2$ metal frame which was placed over the plot to mark the edges and the diver estimating the cover visually. The cover of all layers of macroalgae (canopy and understory of all heights) was estimated, and therefore total % cover could exceed 100%.

Data analysis

Univariate analyses were conducted on the percentage cover of key structural algal species and groupings measured at the end of the experiment using a 3-way Analysis of Variance (ANOVA) with fixed-effect factors of ‘sediment’, ‘nutrients’ and ‘urchins’, each with 2 levels, i.e. ‘ambient’ and ‘enhanced’. Key structural algae analysed by univariate statistics were *E. radiata*, all furoid species combined (*Sargassum vestitum*, *Sargassum decipiens*, *Sargassum fallax*, *Cystophora moniliformis* and *Cystophora monilifera*), all fleshy understorey species (excluding turf-forming), and turf-forming algae. All univariate statistical analyses were undertaken using R (The R Foundation for Statistical Computing, Version 2.15.1, © 2012). Data were checked for conformity to the assumptions of homoscedasticity and normality. Where data were heteroscedastic, the transformation used to stabilise variances was determined using the *Box-Cox* procedure (available in the MASS package). Any transformations used are reported in the relevant figure and table captions, or within the tables. Multiple range tests were conducted using the Tukey’s (honest significant difference) procedure in R. To test the multivariate ‘community response’ of all algal species simultaneously, a Permutational Multivariate Analysis of Variances (PERMANOVA) was conducted using the PRIMER software (Version 6.1.12 & PERMANOVA+ Version 1.02, © 2009 PRIMER-E Ltd.). As for the univariate analyses, the multivariate design consisted of three fixed and crossed factors (‘sediment’, ‘nutrients’ and ‘urchins’), each with 2 levels (‘ambient’ and ‘enhanced’). Patterns in multivariate space were depicted in an ordination plot from a canonical analysis of principal coordinates as a graphical overview of the structure of data and separation of the treatments.

Results

Effects of stressors on canopy-forming algae

Over the 14 months of the experiment, the cover of all canopy-forming macroalgae initially declined over the summer in all treatments (measured after 3 and 6 months), but then increased more rapidly in the presence of nutrients and / or sediments than in ambient ‘control’ plots. Kelp declined in any treatment subject to urchin enhancement (Fig. 2). After 14 months of treatments, the cover of kelp *E. radiata* responded significantly to both nutrient

and sea urchin enhancement. Nutrient enhancement had a positive ‘bottom-up’ effect on kelp cover, while urchin enhancement had a significant negative ‘top-down’ effect (mean cover of ~ 35% and 2% cover respectively compared to 8% cover in control plots under ambient conditions (Fig. 3a, Table 2). Pairwise comparison (Tukey’s HSD, $p < 0.05$) showed that urchin enhancement resulted in lower kelp cover, which was significantly lower than kelp cover in treatments either with enhanced sediment, or the combined effects of sediment plus nutrient enhancement (Fig 3a). Notably, sediment enhancement had a slightly positive (but non-significant) effect on kelp cover (18% cover compared to 8% cover in control plots, Fig. 3a, Table 2).

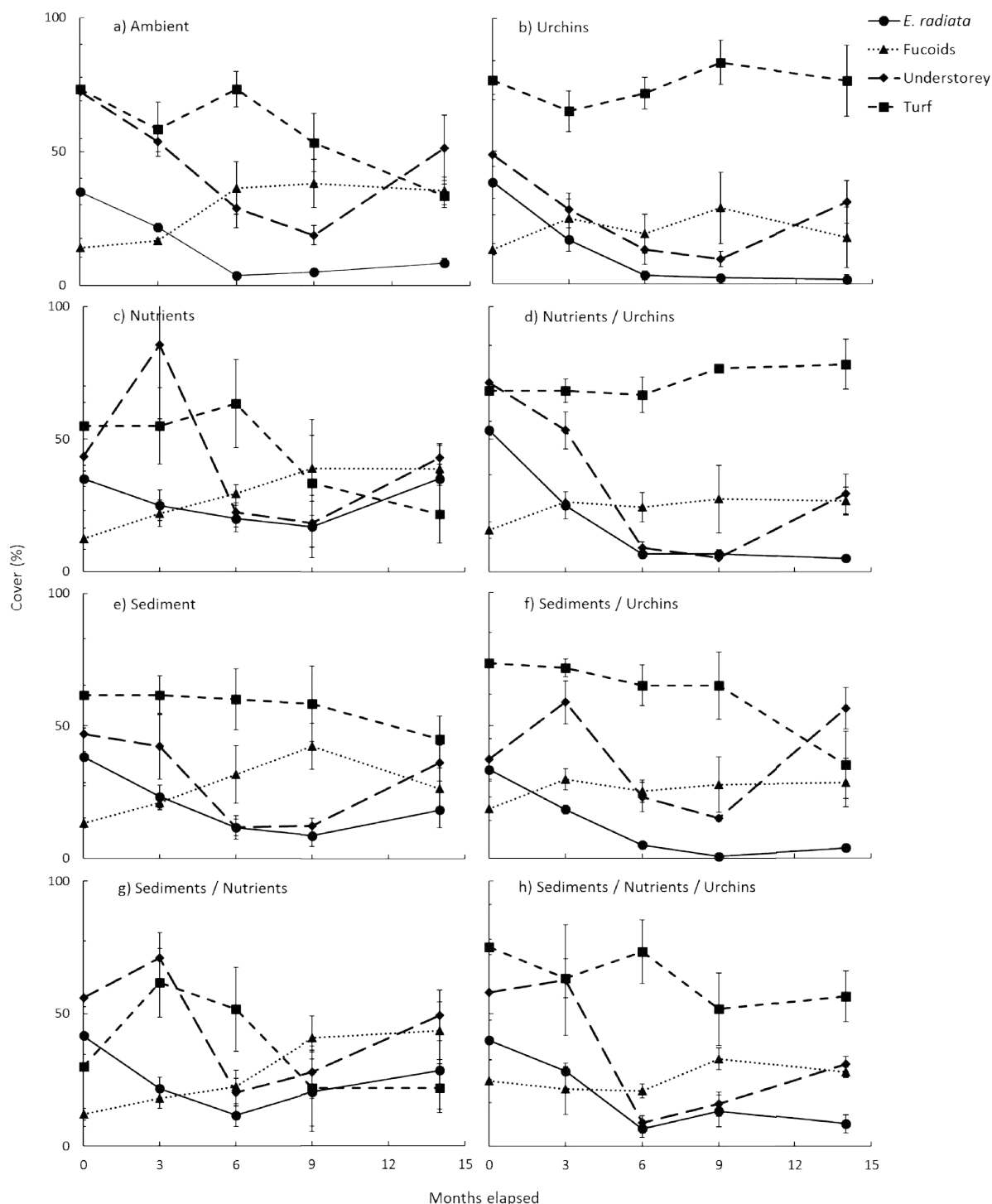


Figure 2. Trends in mean percentage algal cover for each treatment throughout the 14 month experiment; each panel displays % cover of *E. radiata* (circles), fucoid algae (*Sargassum* spp. and *Cystophora* spp., triangles), understorey-forming algae (excluding turf-forming algae, diamonds) and turf-forming algae (squares) for all 8 treatments combination: a) ambient / no enhancements, b) enhanced (= ↑) urchins, c) ↑ nutrients, d) ↑ nutrients plus ↑ urchins, e) ↑ sediments, f) ↑ sediments plus ↑ urchins, g) ↑ sediments plus ↑ nutrients, and h) ↑ sediments plus ↑ nutrients plus ↑ urchins.

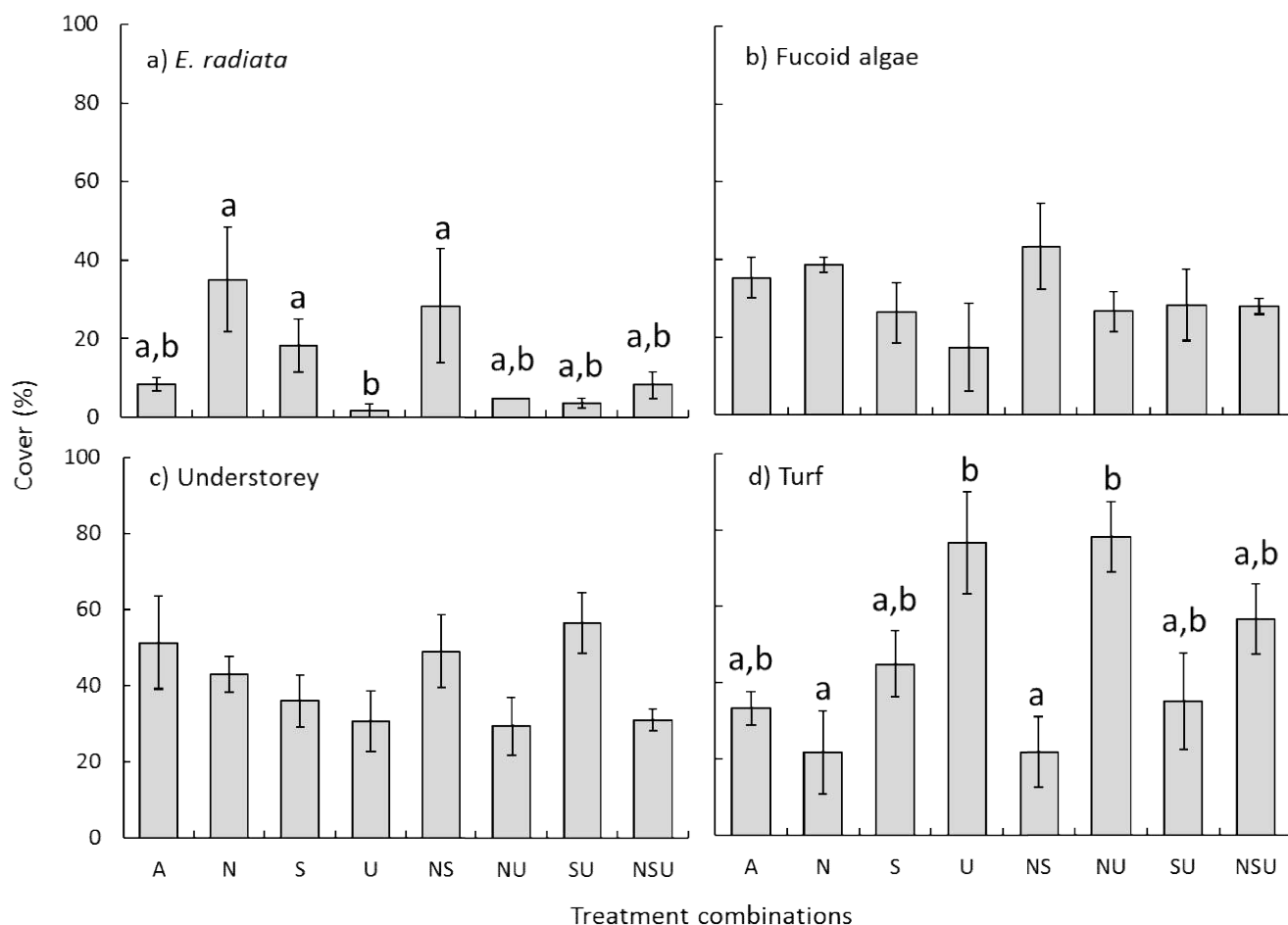


Figure 3. Response in percentage cover of (a) *E. radiata*, (b) Furoid brown algae, (c) Understorey algae (except turf and encrusting algae), and (d) Turf-forming algae at the conclusion of the 14 month kelp bed experiment in south-eastern Port Phillip Bay, December 2012 to February 2014. Treatments are: A = ambient, N = 200 g of fertiliser added every 6 weeks, S = sediment addition at twice the natural deposition rate, U = urchin abundance increased to more than 2 times ambient density; treatments occur in all possible combinations. Letters above bars indicate significant Tukey's HSD groupings ($p < 0.05$).

Cover of furoid species (*Sargassum decipiens*, *Sargassum vestitum*, *Sargassum fallax*, *Cystophora moniliformis* and *Cystophora monilifera*) either maintained or slightly increased their cover through the entire period of the experiment (Fig 2). At the end of the experiment, furoid species alone were not significantly influenced by any of the treatments, but plots with urchin enhancement showed lowest cover (17%, less than half of the cover reported in ambient plots with 35%) which was nearly significant at nominal $\alpha = 0.05$ (p -value of 0.06; see Table 2, Fig 3b). £

Table 2. Results of fixed effects 3-way ANOVAs comparing effects of treatments ('Nutrient', 'Sediment' and 'Urchins', each with 2 levels of ambient and enhanced) on the percentage cover of *E. radiata*, furoid algae, understorey-forming algae (Understorey) and turf forming algae (Turf-formers) at the conclusion of the experiments. Only data for *E. radiata* required transformation ($Y^{0.2}$), while all other response variables showed normality and homoscedasticity. F-values in bold with asterisks indicate significance at levels of $*0.01 < p \leq 0.05$, $**0.001 < p \leq 0.01$, $*** p \leq 0.001$; values in bold without asterisks indicate $p = 0.06$.

| | <i>E. radiata</i> | | | Furoid Algae | | | Understorey | | | Turf-Formers | | |
|------------------|-------------------|-------------|-----------------|--------------|--------|-------------|-------------|--------|-------------|--------------|----------------|-----------------|
| Transformation | $\gamma^{0.2}$ | | | - | | | - | | | - | | |
| Source | df | MS | F | df | MS | F | df | MS | F | df | MS | F |
| Nutrients | 1 | 0.65 | 8.18* | 1 | 322.70 | 1.90 | 1 | 181.50 | 0.97 | 1 | 51.00 | 0.17 |
| Sediment | 1 | 0.12 | 1.47 | 1 | 24.00 | 0.14 | 1 | 121.50 | 0.65 | 1 | 1001.00 | 3.30 |
| Urchins | 1 | 1.58 | 20.00*** | 1 | 704.20 | 4.16 | 1 | 384.00 | 2.06 | 1 | 5859.00 | 19.33*** |
| Nut x Sed | 1 | 0.15 | 1.88 | 1 | 6.00 | 0.04 | 1 | 2.70 | 0.01 | 1 | 26.00 | 0.09 |
| Nut x Urch | 1 | 0.01 | 4.16 | 1 | 48.20 | 0.28 | 1 | 368.20 | 1.97 | 1 | 1276.00 | 4.21 |
| Sed x Urch | 1 | 0.07 | 0.88 | 1 | 104.20 | 0.62 | 1 | 504.20 | 2.70 | 1 | 2109.00 | 6.96* |
| Nut x Sed x Urch | 1 | 0.00 | 0.04 | 1 | 204.20 | 1.21 | 1 | 770.70 | 4.13 | 1 | 376.00 | 1.24 |
| Residuals | 16 | 0.08 | | 16 | 169.40 | | 16 | 186.70 | | 16 | 303.00 | |

Effects of stressors on understorey- and turf-forming algae

During the experiment a considerable decrease in understorey cover was observed at 6 and 9 months, but increased at the final assessment across all treatments (Fig. 2). After 14 months, none of the treatments had significant effects on the cover of understorey-forming algae (i.e. all algae excluding canopy forming browns, turf-forming and encrusting algae; see Fig. 3c), but the interaction effect across all treatments on understorey algae (i.e. sediment*nutrients*urchins) was marginally non-significant ($p = 0.06$; see Table 2).

Turf-forming algae did not show the initial overall decline as occurred with canopy-forming and understorey algae, but cover diverged among treatments at 9 months (Fig 2). At the final assessment plots receiving enhanced nutrients showed lower turf cover, except where nutrients were combined with urchin enhancement (Fig. 2c, d, g & h). Sediment enhancement on its own had very little effect on turf cover over the duration of the experiment (45% cover in comparison to 33% found in control plots). Sediment enhancement in combination with

nutrients did not change the negative effect of nutrients (mean turf cover 22% with nutrient enhancement as a single stressor and in combination with sediments), but when in combination with urchins seemed to weaken the positive effect of the urchin enhancement (77% for urchins as single stressor and 35% when in combination). Urchin enhancement on its own and in combination with nutrient enhancement had a significant positive effect on the cover of turf-forming algae after 14 months (77% and 78% respectively, Table 2, Fig. 3d). Pairwise comparison (Tukey's HSD) showed a significantly higher cover of turf algae in plots with urchins, and urchins plus nutrient enhancement, compared to plots with only enhanced nutrients and with added nutrient plus sediment enhancement (Fig. 3d).

Effects of stressors on the algal community structure

After 14 months, 3-way Permutational Multivariate Analysis of Variance (PERMANOVA) showed algal community structure to be significantly different when urchin numbers were enhanced. No other treatment with any other factors singly or in combination revealed differences in community composition (see Fig. 4, Table 3).

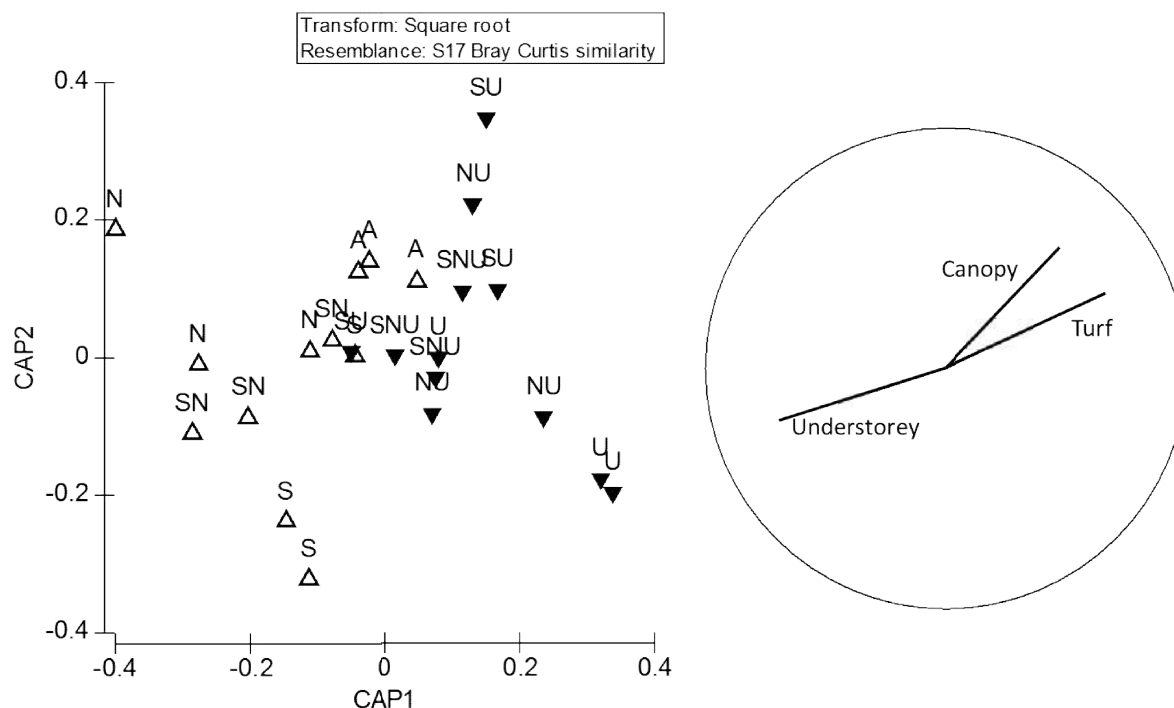


Figure 4. Ordination plot from a canonical analysis of principal coordinates (CAP) based on mean algal community composition for each treatment combination at the end of the 14 month experiment with associated vector biplot (to the right); A = ambient, N = 200 g of fertiliser added every 6 weeks, S = sediment addition at twice the natural deposition rate, U = urchin abundance increased to more than 2 times ambient density m^{-2}). Black triangles are urchin enhancement plots and white triangles are plots with ambient urchin densities ($8.3 \pm 0.3 \text{ m}^{-2}$ and $3.8 \pm 0.2 \text{ m}^{-2}$ respectively).

Table 3. Fixed effects PERMANOVA comparing effects of treatments ('Nutrients', 'Sediments' and 'Urchins' all with 2 levels: ambient and enhanced) on algal community composition after 14 months of treatment. The analysis was conducted on a Bray-Curtis matrix after square root transformation of the raw data. Significant effects highlighted in bold at $\alpha = 0.05$.

| Source | df | MS | F | Unique Perm | P |
|----------------------------|----|--------|------|-------------|--------------|
| Nutrient | 1 | 488.72 | 2.89 | 999 | 0.06 |
| Sediment | 1 | 58.62 | 0.35 | 999 | 0.76 |
| Urchin | 1 | 1478.5 | 8.75 | 999 | 0.001 |
| Nut. x Sed. | 1 | 90.74 | 0.54 | 999 | 0.67 |
| Nut. x Urch. | 1 | 63.77 | 0.38 | 997 | 0.73 |
| Sed. x Urch. | 1 | 482.49 | 2.86 | 999 | 0.06 |
| Nut. x Sed. x Urch. | 1 | 328.07 | 1.94 | 999 | 0.16 |
| Residuals | 16 | 168.9 | | | |
| Total | 23 | | | | |

Discussion

Effects of stressors on canopy-forming algae

Ecklonia radiata was the only algae that benefitted from nutrient enhancement, which is likely due to the dominance of this species over understorey species via shading and abrasion (Connell 2003, Russell 2007). Most other studies examining nutrient effects on canopy-formers have been conducted on the early life stages (Amsler & Neushul 1990, Van Alstyne & Pelletreau 2000, Agatsuma et al. 2014), and the few *in situ* studies that have considered nutrient effects on adult sporophytes mostly show negative or no effect (Pfister & Van Alstyne 2003, Kraufvelin et al. 2010).

In line with many other studies (e.g. Chapman 1981, Dayton 1985, Wright et al. 2005, Ling et al. 2010), our results clearly demonstrate a decline in cover of canopy-forming algae caused by elevated urchin abundance. Cover of *E. radiata* in plots with enhanced urchins was less than half of that observed in control plots experiencing ambient urchin conditions (Fig. 3a), whereas cover of fucoid species were only slightly reduced in plots with enhanced urchin

numbers (Fig. 3b). The more pronounced decline in *E. radiata* compared to fucoids (mainly *Sargassum vestitum*) in the presence of urchins at high density is consistent with findings of other studies suggesting a preference by urchins for laminarian over fucoid species (Hill et al. 2003, Vanderklift & Kendrick 2005). Gut content analyses of urchins in three regions of PPB showed a clear prevalence of brown algae (Appendix, Fig. 1), but distinguishing among different species of browns was not possible from visual analysis. However, sea urchins were often observed clinging to *E. radiata* fronds, weighing them down and feeding on them, but we did not see this behaviour with fucoid species.

The negative effect of grazing on kelp by urchins at elevated densities was observed even when nutrients were enhanced. Thus, where urchin abundance reaches high levels (densities $> 4 \text{ m}^{-2}$, Kriegisch et al. 2016), the reef community is clearly under top-down control, which overwhelms any effect of nutrients to stimulate kelp growth. This is also demonstrated by Guarnieri et al. (2014) who shows that recovery of kelp beds can be achieved by simply removing sea urchins, but recolonisation is ameliorated when nutrients are enhanced. The net effect of the antagonistic actions of elevated urchin density and nutrient concentration (having negative and positive effects on kelp cover respectively) is a new finding for an experimental study, as the combination of nutrient enhancement and urchin enhancement has not previously been studied simultaneously in an experimental setting. Most other research focuses on the combined positive effects of nutrient enhancement and grazer removal (Hauxwell et al. 1998, Worm et al. 1999, Hillebrand 2003, Russell & Connell 2005, Korpinen et al. 2007, Atalah & Crowe 2010), with few studies assessing algal growth when urchin herbivory was reduced and nutrient concentrations increased (e.g. Guarnieri et al. 2014).

The enhancement of sediments to twice the ambient deposition rate did not have any detectable effect on the cover of any canopy-forming algae, neither as a single treatment nor in combination with nutrient or urchin enhancement. This finding contrasts other studies in which sediments have been shown to have great influence on the assemblage of algae on rocky reefs (Airoldi 2003, Valentine & Johnson 2005b, Valentine & Johnson 2005a, Shepherd et al. 2009, Strain et al. 2014). Unfortunately, most studies assessing sediment stress on macroalgae lack information about natural deposition rates and therefore it is difficult to assess any general impacts of increasing sedimentation. However there are a few examples, such as Balata et al. (2007) who increased sedimentation rates by 13.6% relative to ambient while Gorgula and Connell (2004) increased sedimentation by $\sim 39\%$, and both

found an increase in turf-forming algae when sedimentation was increased. More recent work on the effects of sediments on macroalgae include the work of Kawamata et al. (2012) who demonstrated recruitment of *Sargassum duplicatum* on sediment covered substratum, while Shepherd et al. (2009) reported the long-term loss of canopy-forming algae and diversity in general associated with increasing sedimentation. Other studies of the effect of sediments have focussed on the early life stages of canopy forming algae (Isaeus et al. 2004, Irving et al. 2009) and show that sediments negatively influence settlement and recruitment. Our results showing no effect of sedimentation are likely due to the canopy-forming species, and larger sub-canopy understorey algae, sweeping the substratum in surge and re-suspending sediments such that sediments did not directly influence presence of algae. Even though the addition of sediments was undertaken on calm weather days with negligible swell, we acknowledge that it was possible that the added sediments were subsequently resuspended and washed away before they could negatively influence the algal community.

Effects of stressors on turf-forming algae

In contrast to observations on many other temperate reefs (e.g. Worm et al. 1999, Gorgula & Connell 2004), in the current study not only did turf-forming algae not benefit from nutrient enhancement, but added nutrient caused a decline in the cover of turf-forming algae. However, these other studies applied the nutrient treatment to un-colonised surfaces and not to intact kelp bed habitat as was done here. Russell and Connell (2005) showed that the addition of nutrients caused turf-forming algal cover to increase in the absence of *E. radiata*, but the same level of nutrient enhancement did not have any effect on turf cover when *E. radiata* was present. Extending the findings of Russell and Connell's study, the experiment presented here shows a positive response of *E. radiata* to nutrient enhancement and decreased turf consistent with increased dominance of kelp over turfs under an elevated nutrient regime. This suggests that the impact of nutrients on temperate rocky reefs is highly dependent on the current state of the reef, producing divergent outcomes if the reef exists as kelp beds as opposed to reef that is dominated by turf.

This finding is further supported by the observation that turf cover thrived in plots experiencing urchin enhancement since urchin overgrazing of attached canopy-forming algae frees space and opens the canopy for the proliferation of turf. When the top-down / bottom-up

effects of urchin enhancement and nutrient enhancement occurred simultaneously, the net result on the macroalgal community was a decrease in kelp and an increase in turf cover. As outlined above, this suggests that the top-down effect of herbivory acting on *E. radiata* was greater than the bottom-up effect of nutrient stimulation of *E. radiata* cover, with the net result that *E. radiata* was grazed down resulting in an algal community dominated by turf.

Enhancement of sediments alone did not have a detectable effect on the cover of turf-forming algae and, when combined with nutrients, had a negative effect on turf cover as was observed when nutrients were enhanced in isolation of other factors. Interestingly, when sediments and urchins were enhanced simultaneously, the positive effect of urchins on turf was not observed and a similar ‘null response’ of turfs was observed as when urchins were enhanced in combination with sediments and nutrients. Thus, it appears that the overall indirect positive effect of urchin grazing on kelps facilitating increased cover of turf is inhibited when there is an increased sediment load. There was no increase in canopy-forming algae in these plots which might be expected if urchins were avoiding plots with added sediment, although the increased sediment cover might also suppress recruitment of canopy forming species (Umar et al. 1998, Chapman & Fletcher 2002, Fabricius et al. 2015). Non-significant increases in the cover of understory-forming algae (non-turfing species) were observed in plots with enhancement of both urchins and sediment (56% cover), which could also partially explain the lower cover of turf-forming algae in these plots. Avoidance of sediment covered reef by urchins has been shown by Kawamata et al. (2011), but there are likely to be thresholds in the amount of accumulated sediment to elicit these responses. Moreover, our maintenance of constant urchin densities in the current study would be likely to mask any such effects if they occurred.

Temporal patterns of algal cover

Cover of both *E. radiata* and understory algae declined in the winter months before increasing again in spring and summer. This pattern reflects that most algal species show greatest growth in the spring and early summer, and least growth (and greatest erosion) in the winter months (Steinberg 1995, Berglund et al. 2003). It appears that in spring and summer growth rates outweigh loss of biomass, while in winter the system is shaped by grazing and / or erosion. Similar seasonal variations have been identified in *E. radiata* cover in Western

Australia (Bearham et al. 2013, de Bettignies et al. 2015). This pattern is consistent with findings from the northern hemisphere (Cubit 1984, Kirkman 1984, Johnson & Mann 1988, Lotze et al. 2001). Hillebrand (2003) for example found that effects of grazing and nutrients on species richness and evenness varied with season.

Effects of stressors on the algal community

The response of the algal community to the factorial manipulation of nutrients, sediments and urchins also clearly revealed sea urchin grazing to be the major driver of overall community structure. Throughout all treatments, only sea urchin enhancement significantly altered macroalgal community structure. This finding is supported by other studies that have shown strong effects of sea urchin overgrazing (e.g. Lawrence J. 1975, Chapman & Johnson 1990, Wright et al. 2005, Ling 2008), yet the current study demonstrates that even in combination with other stressors, sea urchin herbivory is capable of keeping pace with the stimulatory effect of nutrification on kelps to remain the dominant forcing of reef community composition. Other studies exploring the combined effects of nutrients and grazing on diversity found very complex results, showing opposing effects (Hillebrand 2003) and dependence on background productivity (Worm et al. 2002). Worm et al. (2002) showed that in low nutrient environments, similar to the one of the current study, nutrients led to a greater diversity when grazers were present, but did not have that strong effect in the absence of grazers. No difference in algal community was detected in the present experiment whether nutrients were enhanced or at ambient levels. The experiment conducted here with enhanced grazer abundance showed that the effect was very clear in driving change in the algal community.

Conclusions

The results show that temperate reef ecosystems are influenced by complex interactions between multiple stressors. In PPB the strongest effect is from sea urchin grazing which had an overwhelming negative effect on canopy-forming algae, but a positive effect on turf-forming algae. Nutrients alone had the opposite effect to grazing for both types of algae, directly boosting kelp cover which impacted on cover of turf. However, when occurring in

combination with grazing, both the positive (on kelp) and negative (on turf) effects of enhanced nutrients were overwhelmed. Therefore the stressors of nutrification and grazing acted antagonistically, with the top-down effect of grazing overpowering any bottom-up effect of enhanced nutrients. Sediment enhancement did not have a detectable effect as a single stressor, but was antagonistic to urchin grazing in its effects on turf-forming algae. No evidence of any synergistic or additive effects of any stressor combination was found in this study.

Studies testing the effects of multiple stressors in marine environments are increasing and are helping to identify and quantify the complexity of ecosystems (reviewed by Strain et al. 2014). From the current study it is clear that the top-down force of sea urchin grazing, when urchins reach sufficient densities (Kriegisch et al. 2016) is the major driver of ecosystem dynamics for rocky reefs within the urbanised PPB. Detailed understanding of the population dynamics of the sea urchin *H. erythrogramma* is warranted given the key role of grazing by this species and overwhelming effect this process has on the entire reef community. Exploring local mechanisms of resilience for kelp beds to urchin overgrazing is needed in order to safeguard collapse from this desirable ecosystem configuration to unproductive urchin barrens. Since it is clear that sea urchin grazing is the main driver of this kelp-bed system, further research should now focus on identifying why urchin abundance has increased sufficiently for overgrazing of kelp to occur, and whether human influences on urchin populations such as overfishing of urchin predators (Pederson & Johnson 2006) and / or climate change (e.g. Ling et al. 2009a) would have had an influence.

Chapter 4: Phase-shift dynamics of sea urchin overgrazing on nutrified reefs

Abstract

Shifts from productive kelp beds to impoverished sea urchin barrens occur globally and represent a wholesale change to the ecology of sub-tidal temperate reefs. Although the theory of shifts between alternative stable states is well advanced, there are few field studies detailing the dynamics of these kinds of transitions. In this study, sea urchin herbivory (a ‘top-down’ driver of ecosystems) was manipulated over 12 months to estimate (1) the sea urchin density at which kelp beds collapse to sea urchin barrens, and (2) the minimum sea urchin density required to maintain urchin barrens on experimental reefs in the urbanised Port Phillip Bay, Australia. In parallel, the role of one of the ‘bottom-up’ drivers of ecosystem structure was examined by (3) manipulating local nutrient levels and thus attempting to alter primary production on the experimental reefs. It was found that densities of 8 or more urchins m^{-2} ($\geq 427 \text{ g m}^{-2}$ biomass) lead to complete overgrazing of kelp beds while kelp bed recovery occurred when densities were reduced to ≤ 4 urchins m^{-2} ($\leq 213 \text{ g m}^{-2}$ biomass). This experiment provided further insight into the dynamics of transition between urchin barrens and kelp beds by exploring possible tipping-points which in this system can be found between 4 and 8 urchins m^{-2} (213 and 427 g m^{-2} respectively). Local enhancement of nutrient loading did not change the urchin density required for overgrazing or kelp bed recovery, as algal growth was not affected by nutrient enhancement.

Introduction

The opposing forces of herbivory and primary production play vital roles in shaping ecosystems. It is important, yet challenging, to reveal how combinations of these so-called top-down ‘consumption’ and bottom-up ‘resource productivity’ controls operate to ultimately drive shifts in ecosystems from one configuration to another (Posey et al. 2002, Burkepile & Hay 2006). The effects of these two controlling forces can be complex, even in relatively simple ecosystems, as either can have an overriding effect in determining their structure. The net effects of bottom-up and top-down forces can also result in a change in system configuration when an ecosystem is pushed beyond critical thresholds, whereby it may shift into a different state that is stable under environmental conditions identical to the original. In this type of discontinuous ecological transition, or ‘catastrophic’ phase-shift, the system does not return to its former state once conditions are restored to those prior to the shift, and may not easily shift back under most conditions (May 1977, Scheffer et al. 2001, Beisner et al. 2003, Collie et al. 2004). This type of shift has been implicated in the dynamics of ecosystem collapse in terrestrial (Sternberg 2001, Hirota et al. 2011, Lindenmayer et al. 2011), limnetic (Scheffer et al. 1993, Dong et al. 2002), and marine environments (Fauchald 2010, Osman et al. 2010, Fung et al. 2011, Blamey & Branch 2012, reviewed by Conversi et al. 2015), and is also considered to pose a dire threat for global ecosystems (Rockstroem et al. 2009).

For temperate reef environments, the best studied shift between alternative states occurs when rocky reef ecosystems undergo a phase-shift from productive kelp beds to impoverished sea urchin ‘barrens’ (Lawrence J. 1975, Chapman 1981, Harrold & Reed 1985, Ling 2008). While the phenomenon is well recognised, the quantitative nature of local phase-shift dynamics and underlying resilience of kelp beds is in general poorly understood, as are the particular factors that may counter the potential of urchin grazing so that the risk of catastrophic shifts is reduced (reviewed by Ling et al. 2015). Bottom-up forces like nutrient availability are key factors that have the potential to alter the resilience of marine ecosystems (Russell et al. 2005, Kraufvelin et al. 2009), yet few studies have examined these in concert with grazing pressure (but see Russell & Connell 2005, Korpinen et al. 2007, Guarnieri et al. 2014) and none have explicitly manipulated alternative collapse and recovery pathways of phase-shift under different nutrient conditions to quantify the tipping points.

Although numerous studies have demonstrated the impact of urchin grazing on kelp beds (see Ling et al. 2015 for overview), most studies focussing on the transition between states have

only examined the shift from barrens to kelp beds following removal of urchins (Andrew 1993, Gagnon et al. 2004, Ling 2008, Bonaviri et al. 2011, Poore et al. 2012, Bulleri 2013). Fewer studies have assessed the shift from kelp beds to barrens (Brandt et al. 2012, Feehan et al. 2012) and only rarely have the transitions in either direction been addressed concurrently (see Andrew & Underwood 1993, Hill et al. 2003). Therefore within particular reef systems there remains a distinct lack of understanding of the range of urchin densities (or biomass density) necessary to precipitate shifts in either direction (reviewed by Ling et al. 2015), and of quantitative estimates of the tipping points.

The question of the potential for interaction among urchin density and nutrient levels is important because along many urbanised coasts phase-shifts resulting in loss of kelp beds is commonly reported (Harrold & Reed 1985, Andrew & Underwood 1993). In some cases it has been shown that stressors, such as elevated nutrients derived from urbanisation (e.g. Connell et al. 2008), have a detrimental effect on kelp beds by enabling subordinate understory algal species to dominate. Conversely, the so-called ‘stressor’ of enhanced nutrients may act to stimulate primary production to offset the trophic impact of urchin grazing and thus increase resilience of kelp-based systems.

Here, the nature of the transition between kelp beds and urchin barrens in both directions across a range of urchin densities is assessed experimentally, as is the influence on these transitions of adding nutrients to the water column to facilitate kelp growth (i.e. simulating a local increase in a potential stressor often characteristic of near-shore urbanised coastal systems). Specifically, the following questions are addressed: (1) What sea urchin density causes overgrazing of kelp beds?; (2) What urchin density is required to maintain existing urchin barrens?; and (3) Does elevating nutrient levels change the underlying phase-shift dynamics between kelp beds and sea urchin barrens?

Materials and methods

Study area

Our study was conducted at Williamstown Beach in Port Phillip Bay (PPB), Victoria, south-eastern Australia where kelp beds (dominated by *Ecklonia radiata*; (C. Agardh) J. Agardh 1848, Family: Lessoniaceae) are declining (Jung et al. 2011) and remnant kelp beds are under

risk of transition to sea urchin barrens characterised by a lack of kelp and other macroalgae. The sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846; Family: Echinometridae) represents the most wide-spread and ecologically important herbivore in PPB and demonstrates active overgrazing of kelp beds (Constable 1989, Ling et al. 2010). *H. erythrogramma* occurs throughout PPB and at local scales can occur at high densities (up to ~ 120 individuals m^{-2}).

To facilitate manipulating sea urchin densities and administering elevated nutrient conditions, ‘artificial’ patch reefs were constructed from naturally occurring boulders which were placed on sand substrata adjacent to Williamstown Beach (S $37^{\circ} 52' 10.5564''$, E $144^{\circ} 53' 36.4884''$). The site is characterised by shallow sub-tidal rocky reef with large boulders and cobbles interspersed by sand patches. The majority of the sub-tidal reef area (2 to 5.5 m depth) is *H. erythrogramma* sea urchin barrens, but a small number of remnant kelp patches dominated by *E. radiata* can be found. The macroalgal community consists of up to 95% cover of *E. radiata*, the fucoid *Sargassum vestitum* ((R. Brown ex Turner) C. Agardh 1820, Family: Sargassaceae) and the introduced Japanese kelp *Undaria pinnatifida* ((Harvey) Suringar, 1873, Family: Alariaceae) with sparse understorey growth, while on the shallowest margin of the sub-tidal area (1.5 m), *Sargassum spp.* are the dominant canopy-forming macroalgae.

In the study area the sea urchin *H. erythrogramma* occurred at an average density of 4.6 m^{-2} in local urchin barrens and 5.2 m^{-2} in kelp beds (at a biomass density of 245 g m^{-2} and 539 g m^{-2} respectively). Following pilot trials at this site over 6 months, a large sand patch ($2,040 \text{ m}^2$) surrounded by rocky reef in a depth of 4 m was selected as a suitable habitat upon which to build experimental boulder patch reefs. Pilot trials demonstrated that natural sand barriers were effective in limiting migration of urchins between patch reefs, enabling urchin density treatments to be maintained efficiently. This approach was superior to using artificial fences or cages, which quickly became fouled and required weekly maintenance.

Experimental design and data collection

Experimental patch reefs: Using SCUBA, small boulders and cobbles (0.20 - 0.50 m diam.) were sourced from the adjacent rocky reef and constructed into 28 patch reefs (each $\sim 0.85 \text{ m}^2$ planar area) on the sand patch. Reefs were separated by at least 5 m from each

other and from the surrounding natural reef. This distance was chosen *a priori* to achieve independence between replicate reefs in respect to the nutrient enhancement treatments applied in the experiment (after Worm et al. 2000 and also confirmed for this study, see 'Nutrients' below, Russell et al. 2005). All boulders and cobbles used to construct the patch reefs were scraped clean of foliose macroalgae (except encrusting red algae) and sessile invertebrates before commencing the experiment. A star picket was driven into the substratum in the centre of each reef to provide a reference point for assessments and as a fixture to hold bags containing nutrient. An additional star picket was positioned next to each reef for the purpose of holding an 'onion bag' containing two reproductive *E. radiata* individuals to ensure that *E. radiata* propagules were not a limiting factor for kelp re-establishment across all experimental reefs. The bags were kept clean from epiphytes and *E. radiata* bearing sori continued to grow within the bags, and were exchanged at least every 2 months. The 28 boulder reefs were assigned randomly to a total of 4 treatments representing combinations of 'reef ecosystem state' (kelp beds vs urchin barrens) and 'nutrient' conditions (ambient vs enhanced), and within each treatment the 7 replicate reefs supported urchins at densities of 0, 4, 8, 12, 15, 20 and 24 urchins m⁻².

Reef ecosystem state: Three adult *E. radiata* individuals were transplanted to each appropriate patch reef to simulate the 'kelp bed state', providing a canopy cover of ~ 50% within the internal 0.25 m² of each patch reef. Kelp attached to small boulders were collected from the nearest kelp bed and first placed on the edge of the sand patch to acclimatise. They were left there for 1 week to eliminate any potential dislodgement or damage due to handling before placing them on the prepared boulder reefs. The 'barrens state' consisted of cleaned boulders without the addition of kelp.

Nutrients: A mesh bag (mesh size 1 x 1 mm) with 200 g of slow release fertiliser (Osmocote® Pro 3-4 M, 17N:4.8P:8.3K) was used to enhance nutrients on individual patch reefs (quantity of fertiliser and technique after Worm et al. 2000). Patch reefs with no nutrient enhancement were assigned a mesh bag with small pebbles as procedural control. Mesh bags with fertiliser were exchanged every 6 weeks to ensure constant supply of nutrients. To confirm that nutrient concentrations were significantly enhanced using this procedure, water samples were taken from both 'elevated nutrient' and 'control' patch reefs midway through the period of routine fertiliser application (i.e. 3 weeks after application fertiliser). Samples were taken 30 cm from the nutrient bag with a 60 ml plastic syringe, then filtered and placed

on ice and analysed at the Water Studies Centre (School of Chemistry, Monash University, Victoria) for concentrations of nitrogen-oxides, ammonia and filterable reactive phosphorus (Table 1). Nutrient concentrations across ‘ambient’ vs ‘nutrient enhanced’ reefs were compared using one-way analysis of variance (ANOVA), with 3 replicates for each level. Analysis showed significant enhancement of concentrations of ammonia and all nutrients combined (1-way ANOVA, $F_{1,4} = 10.76$, $p < 0.05$ and $F_{1,4} = 18.86$, $p = 0.01$ respectively; Table 1).

Table 1. Mean values and results of 1-way ANOVAs of nutrient concentrations for ‘Ambient’ and ‘Enhanced’ nutrient conditions on experimental patch reefs. Mean values ($\mu\text{mol l}^{-1} \pm \text{SE}$, $n = 3$) and results of ANOVAs are shown for ammonia (NH_3), nitrate and nitrite combined (NO_x), filterable reactive phosphorus (FRP) and the sum of all nutrients (Total). Values in bold are significant at $\alpha = 0.05$. The transformation of Y^{-9} was used for FRP, none of the other concentrations needed a transformation.

| | Ambient | Enhanced | F | <i>p</i> |
|-----------------------|---------------------|---------------------|-------|------------------|
| NH₃ | 0.27 (± 0.07) | 0.69 (± 0.1) | 10.76 | < 0.05 |
| NO_x | 0.04 (± 0.01) | 0.06 (± 0.01) | 4.00 | 0.12 |
| FRP | 1.56 (± 0.03) | 1.66 (± 0.07) | 1.82 | 0.25 |
| Total | 1.88 (± 0.06) | 2.41 (± 0.11) | 18.86 | 0.01 |

Sea urchins: After randomly assigning the 4 different ‘reef state’ and ‘nutrient condition’ treatments to the experimental patch reefs, each of the 7 replicate patch-reefs within each treatment received (at random) one of 7 different urchin densities (0, 4, 8, 12, 15, 20 and 24 urchins m^{-2}). Reefs were then checked every 3 weeks for the 12 months of the experiment to ensure urchin densities were maintained at their designated level. The range of urchin densities selected was based on surveys conducted on reefs across northern PPB, which showed mean urchin densities of 4.6 ± 0.5 urchins m^{-2} (245 ± 45 g m^{-2} , $n = 25$ random $1 \times 1 \text{ m}^2$ quadrats) and 5.2 ± 0.7 urchins m^{-2} (539 ± 80 g m^{-2} , $n = 25$ random $1 \times 1 \text{ m}^2$ quadrats) for sea urchin barrens and kelp beds respectively. Thus, urchin densities chosen for the

experiment ranged between 0 to ~ 5 times the mean observed densities for barrens and 0 to 4.5 times the mean for reefs starting as the ‘kelp bed’ state.

Assessments

The experiment was run for ~ 13 months, from November 2012 to December 2013, and the amount of erect macroalgae on each reef was assessed every 3 months. Assessments were undertaken on SCUBA with *in situ* estimates of percentage cover of canopy-formers and understorey. Percentage cover assessments were performed using the point-intercept method (a 50 by 50 cm quadrat with 49 regularly spaced points defined by intersecting string lines, and an additional random point within the quadrat assigned in advance). Stipe counts were used to enumerate the abundance of canopy-forming brown algae (*E. radiata*, *S. vestitum*, and the introduced ephemeral Japanese kelp *U. pinnatifida*), and on the final assessment the total number of macroalgal species on each patch reef was counted.

Statistical analysis

The effect of ‘reef state’ and ‘nutrient conditions’ on canopy-forming algae was assessed across the gradient of sea urchin densities. The analyses were undertaken in two parts; initially the rate of shift between reef states (in both directions) was investigated by analysing the planar % cover of canopy-forming algae with a 1-way Model I Analysis of Covariance (ANCOVA) using the factor ‘nutrients’ (2 levels: ambient *vs* enhanced) and with sea urchin density as the covariate. Second, macroalgal species richness and diversity (Shannon-Wiener Index) at the end of the experiment was calculated and analysed using a 2-way Model I Analysis of Covariance (ANCOVA) with factors ‘reef state’ (2 levels: kelp *vs* barrens) and ‘nutrients’ (2 levels: ambient *vs* enhanced), with sea urchin density as the covariate. Because the data contained some zeros due to complete overgrazing or lack of kelp recovery, the assumption of homoscedasticity was violated (slightly) in some cases even after transformation of the data, but since the design was balanced the impact of the violation on error rates and power is unlikely to be problematic. The transformation to stabilise variances was determined using the *Box-Cox* procedure. *RStudio* (Version 0.98.953 - © 2009-2013 *RStudio, Inc.*) was used for all statistical analyses.

Results

Kelp bed overgrazing

Reefs initiated in the ‘kelp bed’ state demonstrated rapid decline in kelp cover when subject to high densities of grazing urchins (Fig 1a and b). The higher the sea urchin density, the more rapid the loss of kelp (Fig 1a and b). Decline in kelp cover under moderate to high urchin densities (12 – 24 urchins m^{-2}) occurred within 3 months (Fig 1a and b; Table 2). After 13 months, any patch reef initiated as a ‘kelp bed’ exposed to urchin densities of $\geq 8 \text{ m}^{-2}$ (an equivalent urchin biomass density $\geq 427 \text{ g m}^{-2}$) was completely denuded of kelp. Conversely, intact kelp remained on reefs with $\leq 4 \text{ urchins m}^{-2}$ (an equivalent biomass density $\leq 213 \text{ g m}^{-2}$) (Fig 3a). This clear response of kelp cover to sea urchin density was independent of nutrient levels (Table 2).

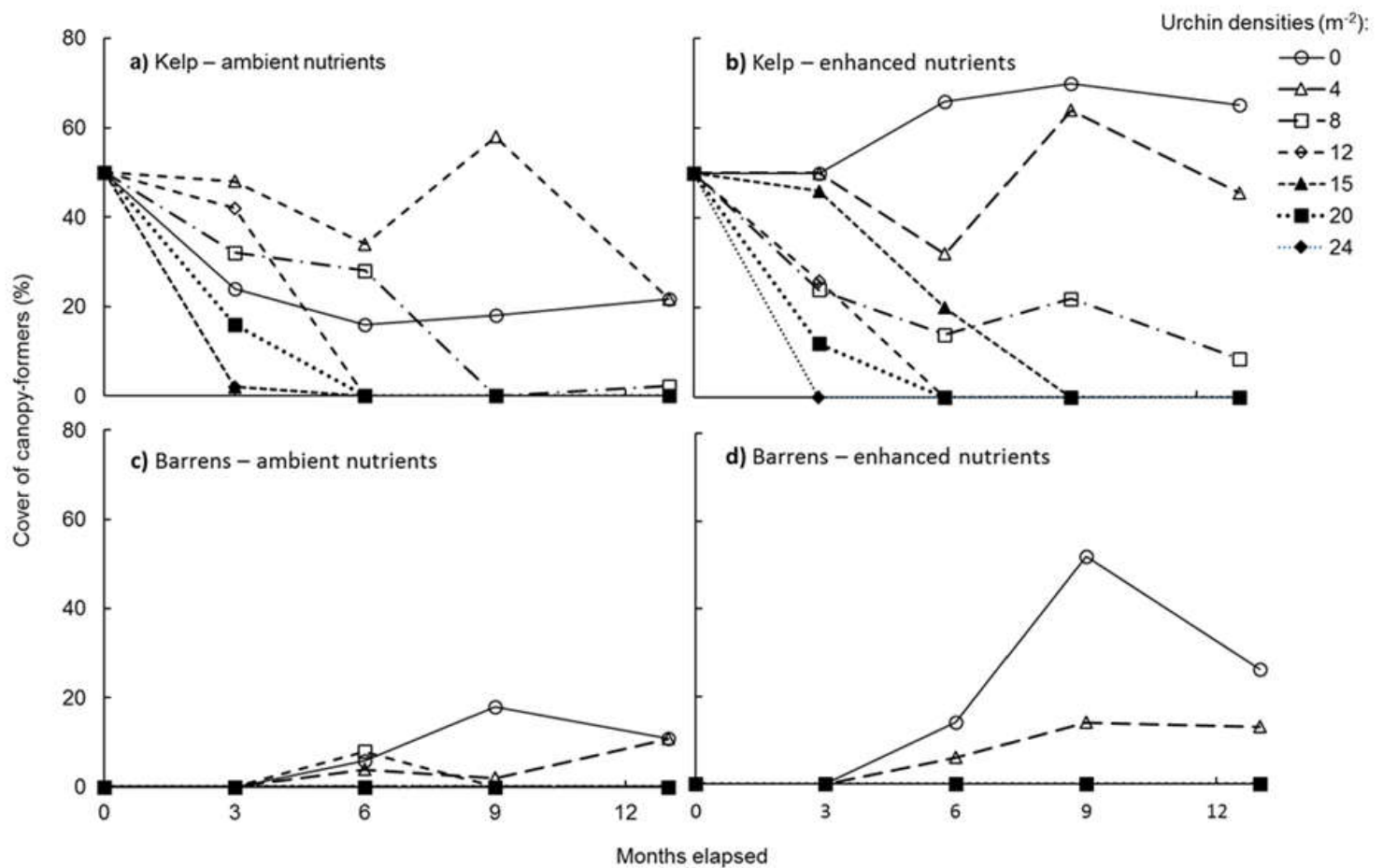


Figure 1. Kelp collapse and recovery at different urchin densities over time. Percentage cover of canopy-forming algae at a range of sea urchin densities (individuals m⁻²) on patch reefs initiated as the ‘kelp bed’ (a) and (b), and ‘barrens’ state (c) and (d) in northern Port Phillip Bay, Nov. 2012 to Dec. 2013. Plots (a) and (c) represent reefs with ambient nutrient levels, and (b) and (d) show results for reefs with enhanced nutrient levels.

Table 2. Results of 1-way fixed effects model I Analysis of Covariance testing the differences in cover of canopy-forming macroalgae at different urchin densities (covariate) and nutrient conditions (ambient *vs* enhanced) for the 4 periods of *a priori* interest across a quarter (3 months), half (6 months), three quarters (9 months) and a full year (13 months). The interaction term (nutrients*urchins) is the test for homogeneity of slopes. (a) Results of reefs starting as the ‘kelp bed state’, and (b) results of reefs starting as the ‘urchin barrens state’. Note that no recovery of canopy-formers was observed after 3 months at any urchin density. Values in bold are significant at $\alpha = 0.05$. Transformations used for overgrazing (a) were $\ln(Y)$ for the assessment at 6 months, and $Y^{-0.5}$ for the assessment at 9 months, while no transformations were used for data collected at 3 and 13 months. For the recovery (b), data were transformed as $\ln(Y)$ for the assessment at 9 months, while a transformation was not required for the other assessments.

| | After 3 months | | | | After 6 months | | | | After 9 months | | | | After 13 months | | | |
|-----------------------|----------------|--------|-------|------------------|----------------|--------|-------|-------------------|----------------|--------|-------|-------------------|-----------------|--------|-------|-------------------|
| | df | MS | F | p | df | MS | F | p | df | MS | F | p | df | MS | F | p |
| a) Overgrazing | | | | | | | | | | | | | | | | |
| Interactive term | 1 | 43.70 | 0.23 | 0.64 | 1 | 0.008 | 0.006 | 0.94 | 1 | 0.16 | 0.19 | 0.68 | 1 | 0.01 | 0.03 | 0.88 |
| Urchins (cov) | 1 | 2409.9 | 13.83 | < 0.01 | 1 | 26.26 | 22.56 | < 0.001 | 1 | 19.50 | 24.60 | < 0.001 | 1 | 27.65 | 39.51 | < 0.001 |
| Nutrients | 1 | 126.00 | 0.72 | 0.41 | 1 | 0.98 | 0.84 | 0.38 | 1 | 0.67 | 0.85 | 0.38 | 1 | 0.60 | 0.86 | 0.37 |
| Residuals | 11 | 174.27 | | | 11 | 1.16 | | | 11 | 0.79 | | | 11 | 0.70 | | |
| b) Recovery | | | | | | | | | | | | | | | | |
| Interactive term | - | - | - | - | 1 | 14.00 | 1.68 | 0.22 | 1 | 284.46 | 2.33 | 0.15 | 1 | 44.34 | 1.48 | 0.25 |
| Urchins (cov) | - | - | - | - | 1 | 115.35 | 13.05 | < 0.01 | 1 | 14.52 | 16.39 | < 0.01 | 1 | 456.23 | 14.57 | < 0.01 |
| Nutrients | - | - | - | - | 1 | 7.14 | 0.81 | 0.39 | 1 | 0.50 | 0.56 | 0.47 | 1 | 21.60 | 0.69 | 0.42 |
| Residuals | - | - | | | 11 | 8.84 | | | 11 | 0.89 | | | 11 | 31.33 | | |

Kelp bed recovery

On patch reefs initiated as urchin barrens, recovery of kelp after 6 months was only observed for sea urchin densities $\leq 4 \text{ m}^{-2}$ (equivalent biomass density $\leq 213 \text{ g m}^{-2}$). Kelp cover increased on reefs with low urchin densities, however no kelp growth was observed on reefs with urchin densities $\geq 8 \text{ m}^{-2}$ (equivalent biomass density of $\geq 427 \text{ g m}^{-2}$) (Fig 1c and d). Analysis of the effects of ‘reef ecosystem state’ and ‘nutrients’ on the response of kelp cover over the range of sea urchin densities examined showed clearly that the only factor influencing regrowth of kelp canopy was sea urchin density, with nutrient levels having no

detectable effect on canopy cover (Table 2). After 13 months, canopy species recovering on reefs starting as the ‘barrens state’ were *S. vestitum* and *U. pinnatifida*. On reefs starting in the ‘kelp bed state’, 14 *E. radiata* recruits were found after 13 months, with 13 of these kelp recruits observed on reefs where adult *E. radiata* were still present at the end of the experiment (i.e. on reefs that were below the ‘recovery threshold urchin density’ of 4 urchins m^{-2} , Fig 2). Only a single recruit was found on a reef which started in the ‘kelp bed state’, but which supported urchins at a density above this threshold. Six kelp recruits were found on nutrient enhanced reefs and 8 on reefs experiencing ambient nutrient conditions.

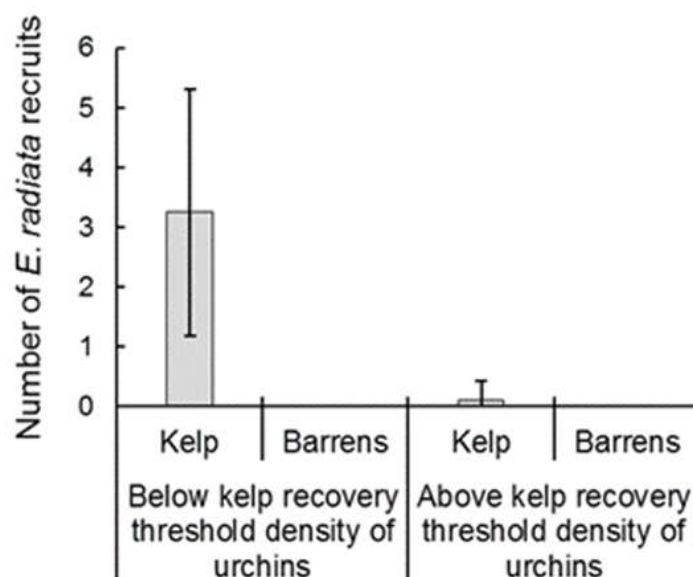


Figure 2. *E. radiata* recruitment. Abundance (mean \pm SE) of *E. radiata* recruits on reefs above and below the critical urchin density (4 urchins m^{-2}) for kelp recovery after 13 months (‘Kelp’ and ‘Barrens’ refers to the initial states of reefs). Nutrient enhancement did not influence recruitment and therefore data for ‘nutrient enhanced’ and ‘ambient nutrient’ reefs were pooled for display.

Algal community response

Examination of the community composition of fleshy algae growing on experimental patch reefs at 13 months (2-way fixed effects ANOVA on species richness and Shannon Diversity) revealed no overall effect of nutrients, while the starting state of the reef and sea urchin

density significantly influenced algal cover (Fig 3b and c, Table 2). Increasing sea urchin densities resulted in declining macroalgal species richness and diversity while, unsurprisingly, reef patches initiated in the 'kelp bed state' showed higher algal species richness and diversity. Yet, as was evident for *E. radiata*, a precipitous decline in species richness occurred at urchin densities between 4 and 8 m⁻² (Fig 3a and b). Interestingly, algal species richness as well as Shannon diversity was highest at 4 as opposed to 0 urchins m⁻². The impoverished algal community on reefs with ≥ 12 urchins m⁻² chiefly consisted of the early colonising species *Ulva sp.* and the ephemeral *Stenogramme interrupta* ((C. Agardh) Montagne) which rapidly monopolise available space when grazing pressure is low.

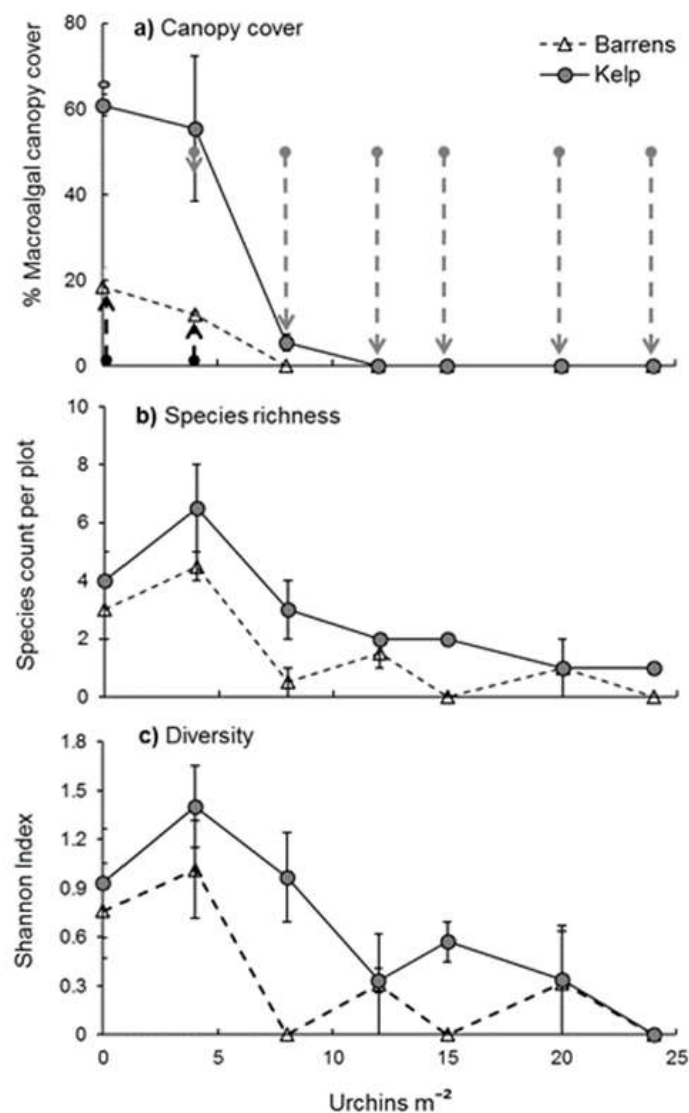


Figure 3. Dependence of algal species richness and diversity on canopy cover. Panel a) details cover of canopy-forming algae, b) shows macroalgal species richness, and c) macroalgal species diversity, against sea urchin densities at the end of the 13 month experimental period. Reefs with initial states of ‘kelp bed’ and ‘urchin’ barrens’ are indicated by the symbols ● and △ respectively; data are means \pm SE. Reefs with enhanced nutrients have been pooled with reefs with ambient nutrient conditions since the addition of fertiliser did not influence response variables (see Tables 2 & 3). Note that species present at the start of the experiment (*E. radiata* for kelp bed reefs and encrusting red algae for all reefs) were excluded from the analysis. Arrows in (a) show responses to experimental manipulation of sea urchin biomass in kelp beds (thick grey arrows = forward-shift ‘collapse’ from kelp to urchin barrens) and on sea urchin barrens (thin black arrows = reverse-shift ‘recovery’ from urchin barrens back to kelp beds).

Table 3. Results of 2-way fixed effects model I Analysis of Covariance testing the significance of differences in responses of species richness (a) and Shannon diversity (b) across different urchin densities (covariate), dependent on initial ‘reef state’ (kelp vs barrens) and nutrient conditions (ambient vs enhanced) after 13 months of treatment. The combined interactions with the covariate (urchins * reef state + urchins * nutrients + urchins * reef state * nutrients) tests for homogeneity of slopes. Values in bold are significant at $\alpha = 0.05$. Transformations used were $Y^{0.45}$ for species richness (a) and $Y^{0.5}$ for Shannon diversity (H).

| | df | MS | F | p |
|----------------------------------|------|-------|---------|-------------------|
| a) Species count | | | | |
| Interactions with covariate | 1.20 | 0.29 | 1.58 | 0.23 |
| Urchins (cov) | 1 | 5.10 | 29.64 | < 0.001 |
| Reef state | 1 | 1.63 | 9.50 | < 0.01 |
| Nutrients | 1 | 0.006 | 0.04 | 0.85 |
| Reef state x Nutrients | 1 | 0.27 | 1.54 | 0.23 |
| Residuals | 23 | 0.17 | | |
| b) Shannon diversity (H') | | | | |
| Interactions with covariate | 1.20 | 0.03 | 0.53 | 0.67 |
| Urchins (cov) | 1 | 1.37 | 23.01 | < 0.001 |
| Reef state | 1 | 0.37 | 3.19 | < 0.05 |
| Nutrients | 1 | 0.00 | < 0.001 | 0.98 |
| Reef state x Nutrients | 1 | 0.07 | 1.22 | 0.28 |
| Residuals | 23 | 0.06 | | |

Discussion

Tipping points of kelp bed collapse and recovery

In this study the density of the sea urchin *H. erythrogramma* required to overgraze kelp on experimental patch reefs was ≥ 8 urchins m^{-2} (equivalent urchin biomass density of 427 g m^{-2}). In contrast, kelp recovery on patch reefs initially starting as ‘barrens’ occurred at urchin densities $\leq 4 \text{ m}^{-2}$ (equivalent biomass density of 213 g m^{-2}). These observations might be interpreted as the existence of a single tipping point between 4 and 8 urchins m^{-2} . However, consistent with other observations worldwide (Ling et al. 2009a) it is more

probable that there are different tipping-points in *H. erythrogramma* density that either drive destructive overgrazing or enable kelp bed recovery, and thus indicate a hysteresis in this system (reviewed by Filbee-Dexter & Scheibling 2014, Ling et al. 2015). The hysteresis would then be found in between 4 and 8 urchins m^{-2} (equivalent to 213 and 427 g m^{-2} urchin biomass density respectively) with recovery closer to 4 and overgrazing closer to 8 urchins m^{-2} .

Many previous studies have demonstrated that sea urchins cause overgrazing and that kelp bed recovery is possible by simply removing urchins from the system (e.g. Lawrence J. 1975, Ortega-Borges et al. 2009, Ling et al. 2010). However, in these ‘all or nothing’ studies, gradients in the density of sea urchins are not explored and typically only a single initial state (i.e. urchin barrens) is considered so that tipping-points that precipitate phase-shift in either direction are elusive. Few studies have ever examined both the response of kelp beds and urchin barrens to gradients in sea urchin density (Andrew & Underwood 1993, Benedetti-Cecchi et al. 1998, Hill et al. 2003, Wright et al. 2005). To date, Hill, Blount et al. (Hill et al. 2003) performed the most detailed experiments on density-dependent urchin overgrazing by separating ‘collapse’ and ‘recovery’ shifts. However, they did not estimate transition thresholds and the recovery and overgrazing experiments were conducted as separate experiments at marginally different scales, in different locations and using different urchin densities across ‘kelp bed’ and ‘barrens’ reef states, so it is difficult to identify hysteresis from their experiments.

Additionally, most studies using sea urchin manipulations report sea urchin density, but not the biomass required to shift from kelp to barrens or *vice versa*. While Filbee-Dexter and Scheibling (2014) and Ling, Scheibling et al. (Ling et al. 2015) both give estimates of densities and biomasses for tipping-points derived from studies worldwide, there remains a lack of definitive studies within particular systems that determine local phase-shift dynamics. It is important to acknowledge that urchins of the same species not only occur at highly variable densities across different sites, but their size structure and thus biomass can also differ substantially among sites (Ling et al. 2015). Only by considering biomass density (g m^{-2}) can variability in the abundance and size of herbivores be taken into account to estimate tipping-points across grazer-driven systems, thus enabling meaningful comparison between different study areas, times and species. Furthermore, it is important to note that the mean urchin biomass measured within remnant kelp beds in northern PPB was $\sim 539 \text{ g m}^{-2}$

which is 112 g greater than the experimentally estimated tipping-point in urchin biomass of kelp bed overgrazing. This observation is consistent with the observed steady decline of remnant beds in this region from 2009 to 2015 (Authors pers. obs.; S. Reeves, unpublished data).

Kelp bed recovery

While kelp was removed relatively quickly at sea urchin densities $\geq 8 \text{ m}^{-2}$, in the treatments where it occurred, the rate of kelp recovery was comparatively slow over the 13 month duration of the experiment. Reductions in urchin densities (usually complete urchin removal) have been shown to be effective in rehabilitating kelp beds in manipulative and ‘natural’ experiments (Johnson & Mann 1988, Shears & Babcock 2002, Ling 2008, Ortega-Borges et al. 2009), however by considering a range of urchin densities this experiment explored possible thresholds of urchin density to allow recruitment and growth of kelp *E. radiata*. Importantly, recruitment was notably greater in the presence of adult sporophytes. This is contrary to the studies of Carnell and Keough (2014) and Flukes, Johnson and Wright (Flukes et al. 2014), who showed greater recruitment of *E. radiata* into patches cleared of canopy-forming algae. There are 2 principal mechanisms that might account for the divergent results among the difference studies. The first addresses the effects of the scale of the kelp bed patch and extent of intraspecific competition. Carnell and Keough (2014) and Flukes, Johnson and Wright (Flukes et al. 2014) worked in large beds of dense kelp where the benthos was heavily shaded (>100% canopy cover) and there was (ostensibly) an abundance of spore production. In contrast, since the small patch reefs initiated in the ‘kelp bed state’ supported only 3 adult sporophytes defining 50% canopy cover, light levels on these patches were likely higher than on larger kelp patches with a similar density of individuals (C. Layton, unpublished data), and thus the effects of intraspecific competition are likely much reduced relative to larger kelp bed patches. Secondly, it is likely that there is an interaction between the timing of the availability of substratum to which new sporophytes can recruit, and the effect of adult kelps sweeping the substratum in surge. Kennelly (1987) showed that timing of canopy clearance has a large influence on recruitment of *E. radiata*, and both Carnell and Keough (2014) and Flukes, Johnson and Wright (Flukes et al. 2014) cleared patches within established kelp canopies just before the seasonal peak (during autumn) in *E. radiata* sorus development and spore release. Studies with other kelps have also clearly demonstrated that the timing of

canopy disturbance relative to seasonal availability of spores is a major determinant of recruitment (e.g. Dayton & Tegner 1984, Johnson & Mann 1988, Chapman & Johnson 1990, Valentine & Johnson 2003). In the current study, experimental reefs were constructed in late spring and therefore were overgrown by understorey algae before *E. radiata* was reproductive, which is likely to have affected the ability of zoospores to settle and gametophytes and sporophytes to develop and gain a foothold on the reef (Kennelly 1987). Given the higher rate of kelp recruitment on patch reefs supporting adult sporophytes compared to those devoid of kelp, it appears that the presence of adult *E. radiata* can enhance recruitment not only through supplying a local source of propagules (which was controlled across all experimental reefs), but also by influencing the surrounding benthos. A likely mechanism is that the sweeping action of *E. radiata* fronds on the substratum as a result of water motion (Kennelly 1989, Russell 2007) reduces the abundance of other algae (particularly turf-forming species) that compete with kelp sporelings or gametophytes. In the current experiment cover of turf-forming algae on experimental patch reefs declined with increasing cover of adult *E. radiata* sporophytes (Fig 4). Overall, these results suggest that rehabilitation of kelp beds on urchin barrens will be most readily facilitated by actively transplanting adult kelp, once urchins have been reduced to densities below the recovery threshold. The adult sporophytes therefore appear to play a dual role by providing both propagules and also reducing the cover of competing understorey in the form of turfing algae.

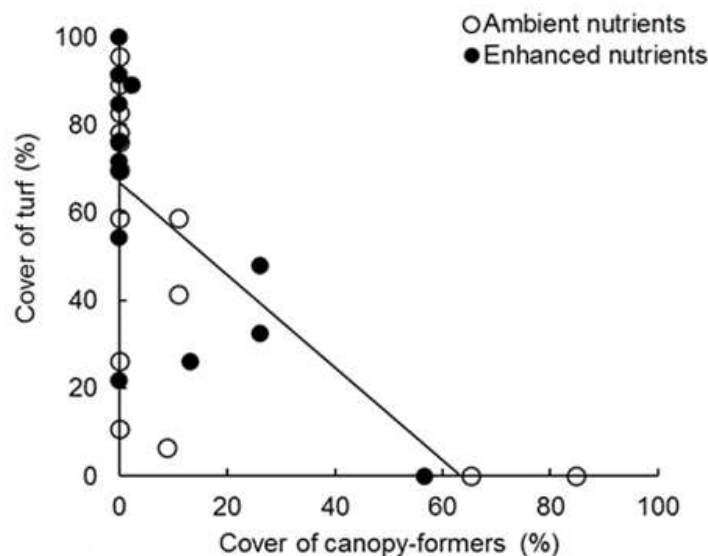


Fig 4. Percentage cover of turf-forming algae *versus* cover of canopy-formers. Open circles display reefs with ambient nutrient conditions and filled circles are reefs with enhanced nutrient levels across all experimental patch reefs at the conclusion of the 13 months experiment (the treatment of ‘reef state’ is not indicated). Fitted line represents treatments pooled across ambient and enhanced nutrient reefs ($R^2 = 0.75$, $y = -0.056x + 4.16$, values derived from linear regression with transformation $\ln(Y)$) because a 1-way ANCOVA showed no significant difference in relationships between reefs with enhanced nutrients and those with ambient nutrient levels (homogeneity of slopes: $F_{1,24} = 0.85$, $p = 0.36$; test between treatments after factoring for the covariate, $F_{1,25} = 1.00$, $p = 0.33$).

Nutrient enhancement

Nutrient measurements for the current study site throughout 2013 showed an average nitrogen concentration (nitrite, nitrate and ammonia) of $0.76 \mu\text{mol l}^{-1}$ (± 0.11), which suggests nitrogen may be limiting algal growth (Chapman & Craigie 1977, North & Zimmerman 1984). This concentration is lower than that reported for other temperate reef systems (e.g. North-West America, Pfister & Van Alstyne 2003, South-West Finland, Korpinen & Jormalainen 2008), slightly higher than published levels for western Australia, and below measurements from New South Wales (Russell et al. 2005). Given this, it was expected that nutrient enhancement would have a strong effect on algal growth. However, nutrient enhancement did not have any detectable effects on either rates of kelp loss or recovery on the experimental reefs. Seeing that the concentrations of ammonia were

significantly higher on reefs with enhanced nutrients but neither phosphorus nor nitrogen-oxides showed higher concentration suggests that at least ammonia was enhanced sufficiently to promote algal growth if this was a limiting factor for production. The technique of nutrient addition was in accordance with other experiments and even exceeded the amount which was added to experimental units in those studies (Worm et al. 2000, Russell & Connell 2005, Russell et al. 2005), so the fact that elevated nitrogen-oxide concentrations were not detected on 'nutrient enhanced' reefs in the current study suggests rapid uptake by microbes, phytoplankton, and microphytobenthos. PPB experiences high rates of denitrification as a result of metabolism of microorganisms (Heggie et al. 1999) and therefore nitrogen-oxides in the water disappear very quickly. This is evident in the discrepancy in the input of nitrogen and what can be found in the water column (Harris et al. 1996). A large sewage treatment plant to the west (i.e. Western Treatment Plant) contributes ~ 3400 tons of Nitrogen year⁻¹ and the Yarra River in the North adds ~ 1050 tons year⁻¹ (Heggie et al. 1999), but concentrations of nitrogen are still low in the water column in PPB (Harris et al. 1996).

Algal community response

Species richness and species diversity of fleshy algae was observed to be highest at an urchin density of 4 m⁻² (equivalent to 213 g m⁻² biomass), which implies that grazing in moderation, or selective grazing on canopy-forming kelp, may release the growth of subordinate understorey algal species leading to a more diverse algal community than in the complete absence of grazing (Menge & Sutherland 1976). *H. erythrogramma* shows preferential grazing on *E. radiata* in eastern Australia (for PPB N. Kriegisch, unpublished data; for NSW see Wright et al. (2005) and Hill et al. (2003)), suggesting that the cause of increased algal diversity at moderate grazing is the result of increased space for the recruitment and growth of less palatable algal species. Due to this selective grazing, this grazer-driven stimulation of both algal diversity and richness may ultimately increase overall resilience of the 'kelp bed' community when kelp production exceeds grazing rates.

Conclusion

Identifying, defining and anticipating critical transitions in nature remain key challenges for understanding and ultimately managing human impacts on natural ecosystems. Here, for the first time, we have used an experimental approach to explore both collapse and recovery dynamics of kelp bed systems prone to sea urchin overgrazing. Consistent with other research, this approach indicates the existence of multiple tipping-points in this system and thus the discontinuous or ‘catastrophic’ nature of transitions between kelp beds and urchin barrens. This study supports the notion that kelp beds and sea urchin barrens exist as alternative stable states of temperate sub-tidal rocky reefs. Furthermore, we have shown that the dynamics of this phase-shift are unaffected by the local enhancement of nutrients, as no evidence was found that the enhancement had any effect on algal production. Importantly, this study has identified the urchin densities at which the ecosystem tipping-points may be found. This information is key for managing the resilience of desirable ecosystem states and for implementing practical measures to avoid being locked into impoverished low-value ecosystem configurations.

Chapter 5: Drift-kelp suppresses destructive grazing by sea urchins

Abstract

Sea urchins have the capacity to destructively overgraze kelp bed habitats and cause a wholesale shift to an alternative and stable ‘urchin barren’ state. However, their destructive grazing behaviour can be highly labile and contingent on behavioural shift at the individual and local population level. Changes in ambient supply of allochthonous food sources, i.e. drift-kelp, is often postulated as a proximate trigger of destructive overgrazing, yet field tests of this hypothesis are surprisingly lacking. Here a suite of *in situ* behavioural experiments were conducted within kelp beds and on sea urchin barrens to test for behavioural switches leading to overgrazing by the Australian sea urchin *Heliocidaris erythrogramma* (Echinometridae). Assays of grazing rates on kelp showed that, on urchin barrens, grazing rates were high on both drift and attached kelp thalli. In contrast, within kelp beds drift-kelp was consumed at high rates, but there was negligible grazing of attached kelp. *H. erythrogramma* moves actively towards and consumes kelp, both within kelp beds and on urchin barrens, but when drift-kelp is supplied, foraging movements across the reef surface are reduced as urchins capture and consume drift-kelp. The collective results provide evidence supporting the notion that destructive overgrazing of attached kelps only occurs once the supply of drift-kelp is interrupted. Furthermore, results indicate that recovery of kelp beds may be possible if a sustained influx of allochthonous food sources were to inundate urchin barrens, particularly where local urchin densities and respective grazing rates may be close to thresholds enabling kelp bed recovery.

Introduction

Herbivory can be a pivotal ‘top-down’ process controlling the abundance of primary producers and consequently the diversity and assembly of plants and animals within an ecosystem (Lubchenco & Gaines 1981). The behaviour of herbivorous animals can therefore be a highly important determinant of ecosystem structure and can be locally diverse and species specific (Ogden & Lobel 1978, Preen 1995, Fox & Bellwood 2013, Tennant & MacLeod 2014). Typically, feeding behaviour shows plasticity, enabling species to cope with spatiotemporal variability in their environment arising from seasonality (Fehmi et al. 2002, Sokos et al. 2015), geographic variability (Lundberg & Golani 1995), and local environmental conditions (Berner et al. 2005).

In sub-tidal marine environments, sea urchins display plasticity in foraging behaviour, sometimes actively overgrazing kelp beds (reviewed by Ling et al. 2015), but at other times and places they inhabit kelp beds at densities sufficient to effect destructive grazing and yet coexist with attached kelps without deleterious effects (Harrold & Reed 1985, Contreras & Castilla 1987, Vanderklift & Kendrick 2004). In these circumstances, a high prevalence of feeding by urchins on detached ‘drift-kelp’ (Harrold and Reed 1985) or on other food sources (salps and diatoms) (e. g. salps, Duggins 1981) advected into the kelp bed has been reported and the role of food availability has been postulated as a proximate driver of sea urchin behaviour (Harrold & Reed 1985, Vadas et al. 1986). Despite previous studies describing shifts in behaviour between ‘active foraging’ on attached kelp vs ‘passive feeding’ on detached ‘drift-algae’ (Dean et al. 1984, Harrold & Reed 1985, Andrew 1993), the causative mechanisms involved in the active switch between behavioural modes, and the effect of any behavioural shift on kelp bed habitat including observed loss of kelp beds across large spatial scales (e.g. Steneck et al. 2002), has not been critically tested.

The behaviourally complex barrens-forming sea urchin *Heliocidaris erythrogramma* (Valenciennes, 1846; Family: Echinometridae) was used in this study to test the underlying mechanisms of overgrazing. Representing one of the most wide-spread and ecologically important species in temperate Australia, it occurs from Shark Bay, Western Australia, through to the central coast of New South Wales and throughout Tasmania (Edgar 1997). It has been observed to display two contrasting modes of foraging: (1) ‘benign’ feeding on drift-kelp (Vanderklift & Kendrick 2005) and (2) active overgrazing of kelp beds (Constable 1989, Ling et al. 2010). Therefore, *H. erythrogramma* possesses similarly labile behavioural

characteristics to the *Strongylocentrotus* genus in the northern hemisphere that forms mobile feeding fronts (Mattison et al. 1977, Lauzon-Guay et al. 2006, Dumont et al. 2007).

Here, a combination of survey and manipulative experiments are used to explore patterns and underlying processes triggering a switch in urchin behaviour, from benign feeding on drift-kelp to active overgrazing of standing kelp beds. To address the role of drift-kelp supply in modulating destructive sea urchin grazing, a suite of field experiments were conducted to address the following sub-questions: (1) In the absence of drift-kelp, does *H. erythrogramma* detect and actively move towards attached kelp?; (2) Does urchin movement differ on urchin barrens vs within kelp bed habitat?; (3) Do feeding rates on drift-kelp vs attached kelp differ across kelp bed and urchin barrens habitat?; and (4) Is there critical change in urchin movements in the presence of drift-kelp?

Materials and methods

Study sites

The study was carried out in the semi-enclosed coastal environment of Port Phillip Bay (PPB) in SE Australia (Fig. 1), where *H. erythrogramma* is the most influential herbivore on shallow rocky reefs and demonstrates both ‘passive’ (feeding on drift-kelp) and active (feeding on attached seaweeds) forms of foraging. To assess urchin foraging dynamics, 4 different experiments were conducted across 4 regions of PPB (Fig. 1). The regions represented a gradient in urchin overgrazing impacts from relatively small barrens patches of 10-100s m² within kelp beds typical of southern regions, to extensive ‘continuous’ barrens stretching over 100,000s m² of sub-tidal reef in northern and western regions. In the north, kelp beds predominantly feature *Ecklonia radiata*, with only sparse growth of furoid species (*Sargassum spp.* and *Cystophora spp.*) whereas in the southern regions kelp beds show more furoid algae and a more developed understory. The underlying reef substratum was consistent across all regions, occurring as mixed boulder / flat rock reef interspersed by sand patches to a depth of 5.5 m.

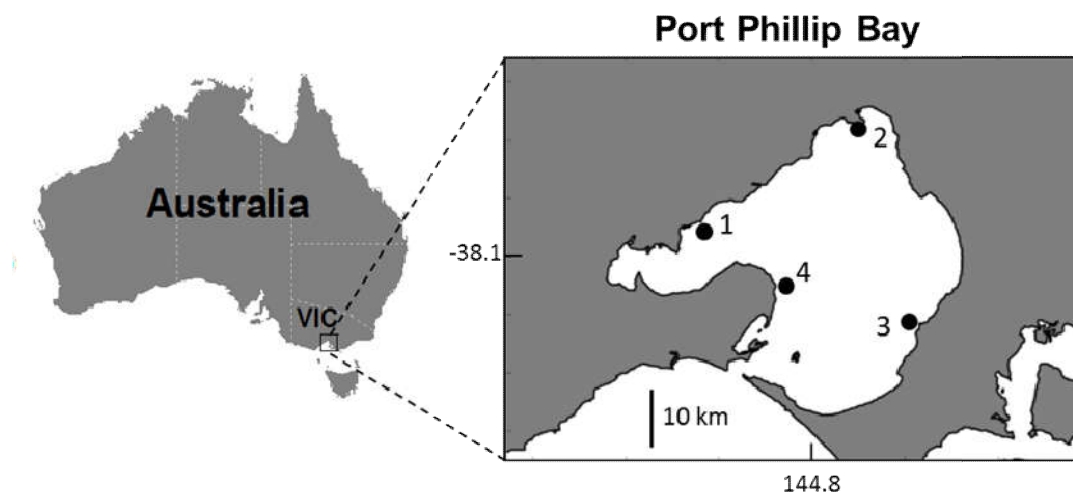


Figure 1. 1: Map of PPB Victoria, southeast Australia. The black dots in the expanded view mark the study site areas: 1 = west, at Kirk's Point (S 38°1'35.742", E 144°34'58.5516"), 2 = north, near Williamstown Beach (S 37° 52' 10.5564", E 144° 53' 36.4884"), 3 = southeast, at Schnapper Point (S 38°12'47.8836", E 145°1'56.517") and 4 = southwest, at Governor Reef (S 38° 9' 8.6106", E 144° 143' 39.5076").

Directional movement of urchins towards attached kelp

Experimental design: This 'choice' experiment was conducted in April 2014 in the north of PPB (Fig. 1, site 2) on barrens habitat (depth range 3 – 4 m). Single urchins, situated on the top of large flat boulders, were randomly chosen for use in the trials. Six dive weights were placed equidistantly at a radius of 200 mm around each selected urchin taking care not to disturb the urchins. The dive weights alternately held down pieces of kelp (*E. radiata*) and surrogate kelp (brown cloth) folded in half in such a way that the two free ends faced the urchin (Fig 2a). The cloth surrogate was similar in structure, colour and size to the *E. radiata* pieces. The dive weights were coated in epoxy and both the weights and surrogate cloth were preconditioned on the sea floor for 2 months prior to start of the experiment to mitigate potentially negative effects of any unknown leachates.

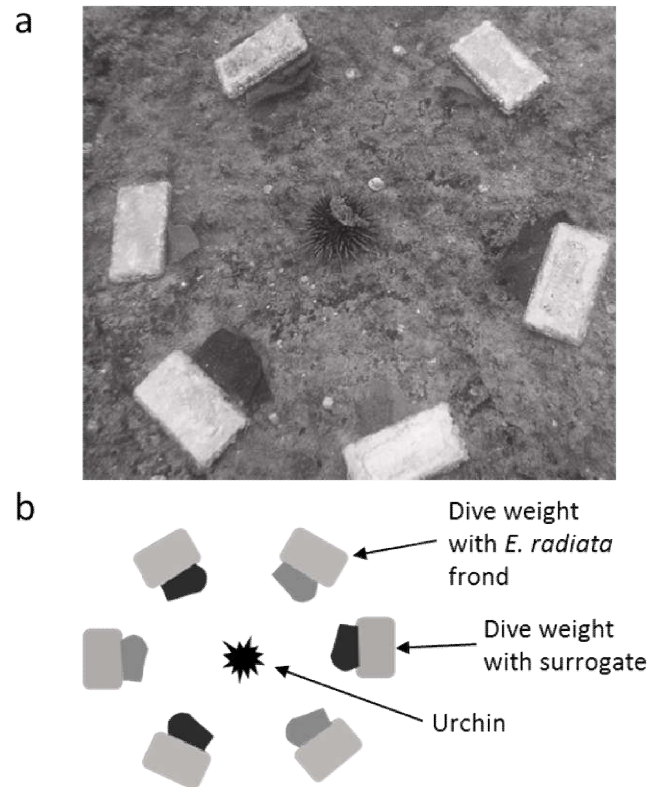


Figure 2. (a) Photo and (b) diagram of the experiment to test for directed movement in sea urchins. Six dive weights surround an individual sea urchin situated on top of a flat boulder with either kelp *E. radiata* or surrogate kelp (brown cloth) underneath each weight (n = 11).

To differentiate urchin choice between natural vs surrogate kelp, the trials were monitored with time-lapse photography. A camera (Panasonic Lumix DCM TS4 in underwater housing) was placed on a 1 to 1.5 m tall tripod fixed to the benthos with tie-downs, and set to take still shots of the trial (Fig 2a & b) every 15 mins over a 15 hour period (pilot trials indicated that a time-lapse frequency of 15 mins sufficiently captured urchin movement dynamics). During trial set-up, individual urchins were neither touched nor moved. A total of 11 replicate trials on different urchin individuals were conducted, all of which were started at midday. A ‘choice’ was recorded when the urchin moved to the kelp or to the surrogate and stayed at that position for more than 30 min (2 consecutive time-lapse images) or alternatively moved entirely out of the field of view, at which point the trial was deemed to have resulted in ‘nil choice’. The kelp *E. radiata* was chosen as it is the dominant canopy-forming species across the range of *H. erythrogramma*, and has been shown to be the urchins’ preferred food (Hill et al. 2003, Vanderklift & Kendrick 2005, Kriegisch unpublished data).

Data analysis: A Chi-Square test was used to test the ‘observed’ vs ‘expected’ frequency of ‘kelp’ vs ‘surrogate’ choices. Statistical analysis for all experiments run in this study were undertaken using R (The R Foundation for Statistical Computing, Version 2.15.1, © 2012).

Movement rates of urchins in different regions and habitats

Experimental design: Movement of *H. erythrogramma* was assessed periodically using time-lapse photography from summer 2012 to summer 2014 in the western, northern and southeastern regions of PPB (Fig. 1, sites 1 - 3). To monitor movement of urchins, 4 time-lapse cameras were used as described above. In this experiment, the frequency of photographs was every 5 minutes and each sequence was 5 hours in duration (i.e. 60 images in total). This was done to adequately capture changes in direction and speed of individual urchins. These sequences were taken in each region, in kelp and barrens habitat if both habitats were present. Six replicate time-lapse sequences were taken in each habitat and in each region during the 2 years (2012 – 2014, $n = 30$ sequences). Each sequence was photographed on a unique patch of reef containing unique urchin individuals to maintain independence of replicate sequences. Criteria for identifying suitable locations were that the reef had low topographic relief with high densities of urchins able to be squarely framed within the camera’s field of view. For each image sequence, an object of known dimensions was photographed to enable calibrating the dimensions of the field of view. Preliminary trials were run at night in the north and southeast in barrens habitat to assess movement across the entire diel cycle. Trials conducted during the day and night (4 replicates for the night and 5 for the day period; $n = 9$) revealed no significant difference of movement between day and night for the different regions (Fig. 3, Table 1a, ‘Diel comparison’). All subsequent monitoring of urchins was therefore performed during daylight hours, which increased ease of camera deployments and retrievals. Furthermore, to account for potential seasonal/temperature variability in urchin movement, initial trials were performed across seasons, but no seasonal effect on urchin movement was detected (linear regression: $R^2 < 0.01$, $F_{1,13} = 0.002$, $p = 0.97$). Once fields of view were calibrated for each image sequence, the ‘Manual Tracking’ plugin of the freeware *ImageJ* (v1.47, National Institutes of Health, USA) was used to process the speed and displacement of urchins (after Flukes et al. 2012).

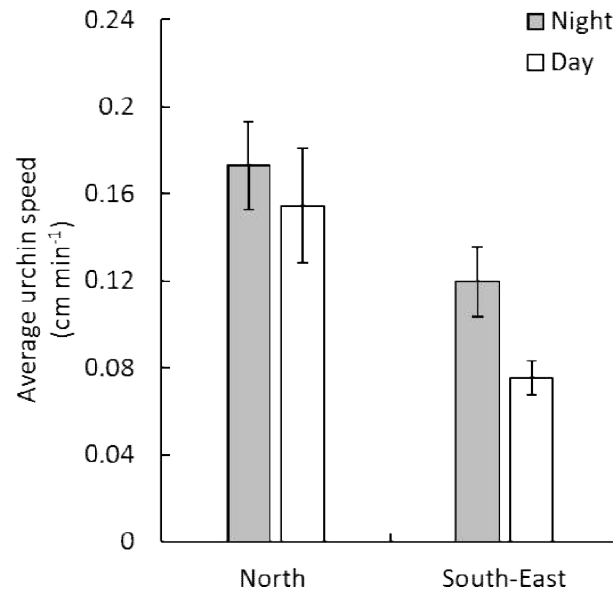


Figure 3. Comparison of average speed of sea urchins in the north and southeast of PPB during the night (grey bars) and day (open bars) with the average speed of each urchin per time-lapse series ($n = 2$, \pm SE).

Data analysis: Images from each time-lapse series were imported to *ImageJ* as an image sequence with area tracked and pixel ratio determined. This enabled the use of the ‘Manual Tracking’ (*ImageJ*) to determine the speed (cm min^{-1}) and net displacement ($\text{cm } 3.75 \text{ h}^{-1}$) of each urchin from image to image, and calculation of average speed and overall displacement for each urchin. The time frame of 3.75 h for displacement was chosen because 95% of the urchins tracked were in the camera field of view for a minimum of 3.75 h. To assess variability in urchin movement rates on barrens habitat across regions within PPB, a 1-way nested Analysis of Variance (ANOVA) was undertaken with factors ‘Region’ (fixed, 3 levels: west, north, southeast) and ‘Plot’ (nested within ‘Region’ with the average speed / displacement of each urchin in 1 time-lapse sequence).

The effect of habitat type on movement was analysed across the north and southeast as barrens and kelp habitat were both present only in these 2 regions. The effect of ‘Habitat’ and ‘Region’ was tested using a 2-way nested ANOVA analysing the factors ‘Habitat’ (fixed, 2 levels: barrens and kelp), ‘Region’ (fixed, 2 levels: north, southeast) and ‘Plot’ (nested within Habitat by Region).

Table 1. ANOVA tests of the differences in sea urchin speed (cm min^{-1}) (i) and net displacement cm 3.75hr (ii) during a) day and night (speed only), b) within different regions of Port Phillip Bay and c) different habitats (kelp beds vs urchin barrens). Transformations used were a) $Y^{0.5}$, b) speed: $Y^{1.3}$, displacement: $Y^{0.1}$, and c) speed: Y^1 , displacement: $Y^{0.4}$; values in bold indicate significant effects at $\alpha = 0.05$. a) shows the effect of region (fixed, north and southeast), time of day (fixed, day and night), and plot (nested within region by time of day) on the speed of sea urchins; b) the effect of region (fixed, west, north and southeast, barrens habitat only) and plot (nested within region) on urchin movement; and c) the effect of habitat (fixed, kelp and barrens), region (fixed, north and southeast) and plot (nested within habitat by region) on urchin movement.

| a) Diel comparison | df | MS | Denominator for F-ratio | F | p |
|---------------------------|-----------|-----------|--------------------------------|----------|------------------|
| Region | 1 | 1.15 | Plot (R x T) | 3.01 | 0.14 |
| Time | 1 | 0.44 | Plot (R x T) | 1.27 | 0.35 |
| Region x Time | 1 | 0.02 | Plot (R x T) | 0.04 | 0.85 |
| Plot (R x T) | 5 | 0.19 | Error | 3.39 | < 0.05 |
| Error | 54 | 0.05 | | | |

| | | | | | |
|-----------------------------|-----|--------|---------------|-------|-------------------|
| b) Region comparison | | | | | |
| i) Speed | | | | | |
| Region | 2 | 415.48 | Plot (Region) | 10.62 | < 0.01 |
| Plot (Region) | 15 | 39.11 | Error | 4.49 | < 0.001 |
| Error | 257 | 8.27 | | | |
| ii) Displacement | | | | | |
| Region | 2 | 35.76 | Plot (Region) | 20.6 | < 0.001 |
| Plot (Region) | 15 | 1.73 | Error | 1.73 | < 0.05 |
| Error | 241 | 0.01 | | | |

| | | | | | |
|------------------------------|-----|--------|--------------|-------|-------------------|
| c) Habitat comparison | | | | | |
| i) Speed | | | | | |
| Region | 1 | 18.25 | Plot (R x H) | 1.99 | 0.17 |
| Habit | 1 | 136.05 | Plot (R x H) | 14.87 | < 0.001 |
| Region x Habitat | 1 | 13.58 | Plot (R x H) | 1.48 | 0.24 |
| Plot (R x H) | 20 | 9.15 | Error | 3.39 | < 0.001 |
| Error | 160 | 2.7 | | | |
| ii) Displacement | | | | | |
| Region | 1 | 6.8 | Plot (R x H) | 4.29 | 0.05 |
| Habit | 1 | 1.86 | Plot (R x H) | 11.83 | < 0.01 |
| Region x Habitat | 1 | 0.11 | Plot (R x H) | 0.7 | 0.41 |
| Plot (R x H) | 20 | 0.16 | Error | 0.16 | < 0.01 |
| Error | 152 | 0.07 | | | |

Kelp consumption rates in different habitats

Experimental design: A standardised assay of sea urchin herbivory was used to compare rates and types of feeding behaviour of urchins on different types of kelp food sources on barrens and in kelp beds, across the 4 different regions of PPB during March 2014. Following the protocol of Vanderklift and Wernberg (2008), assays in kelp and barrens habitats in the same region occurred at a minimum separation distance of 50 m, to ensure the urchin populations were independent based on maximum urchin movement rates over the duration of the assay. Prior to the start of the assays, urchin densities/m² and biomass/m² were defined for each habitat (Table 2) from a sample of 8 randomly chosen replicate quadrats (1 m²). Densities were converted to biomass by estimating the mean test diameter from n = 10 urchins in each region and habitat, and converting this to biomass based on the relationship between test diameter and weight calculated for each region / habitat (this relationship was determined from 48 – 90 urchins sampled randomly). To simulate the different types of kelp food sources (attached kelp on the benthos, drift kelp on the benthos, the canopy of attached kelp elevated away from the substratum, and caged kelp as a control) pieces of kelp were tethered to metal chain in different ways. To mimic attached kelp on the benthos accessible to sea urchins (and any other benthic herbivores), kelp pieces were fastened directly to the chain with clothes pegs. For simulation of drift-kelp, light nylon monofilament fishing line was attached to pieces of chain and a piece of kelp was held in place by a clothes peg at the other end of the fishing line. To examine the amount of fish grazing on kelp, pieces of kelp were fastened as per mimicking drift-kelp, but additionally with a float so that the kelp lamina floated at canopy height and was not accessible to urchins. To account for loss of algae due to tissue degradation and erosion (i.e. the ‘control’ treatment), another set of kelp pieces were fastened directly to the chain and protected with small plastic cages of mesh size 5 mm. Given the short duration of the experiment it was assumed that the lightweight cages did not affect the area of the kelp pieces within them. Each treatment had 12 replicates (48 pieces of algae in total) and the experiment was run for 3 days.

The standardised kelp pieces used in the assays, i.e. 50 mm lengths of clean healthy lateral frond tissue of *E. radiata* with no visible epiphytes, were freshly harvested on the day of the experiment. To determine kelp loss over the duration of the experiment, the kelp pieces were photographed both prior to and at the conclusion of the assay. Kelp pieces were held flat by pressing the sample between a clear Perspex sheet and an opaque sheet and photographed.

The change in kelp area was calculated using the freeware *ImageJ* as a proxy to estimate rates of consumption (the area of the ‘control’ pieces did not change over the course of the experiment). To convert consumption rates as planar area to biomass, 10 kelp sections ranging in size were taken to the laboratory, photographed and wet weights taken in order to calculate biomass per unit area.

Table 2. Density and biomass and the correlation between them (‘Allometric equation’ and ‘ R^2 ’) of *H. erythrogramma* across different regions and habitats of Port Phillip Bay, 2013. Biomass was calculated using allometry derived from measuring the test diameter and weighing n urchins from each habitat and each region. ‘ y ’ refers to the biomass and ‘ x ’ to the test diameter. The allometric equation was used on each urchin to estimate biomass and then the average of these biomasses was reported. Note that R^2 shows the strong correlation between size and weight.

| Region | Habitat | Average urchin density (m ²) | Average urchin biomass (g/m ²) | Allometry equation | R^2 | n |
|------------|---------|--|--|----------------------|-------|-----|
| West | Barrens | 24 | 627.0 | $y=0.0007x^{2.8502}$ | 0.97 | 70 |
| North | Barrens | 5 | 196.7 | $y=0.0013x^{2.6946}$ | 0.96 | 60 |
| | Kelp | 6 | 336.4 | $y=0.001x^{2.7542}$ | 0.97 | 77 |
| South-East | Barrens | 7 | 680.0 | $y=0.0009x^{2.7914}$ | 0.98 | 90 |
| | Kelp | 4 | 556.4 | $y=0.0004x^{3.003}$ | 0.98 | 90 |
| South-West | Barrens | 7 | 506.7 | $y=0.0014x^{2.6849}$ | 0.98 | 48 |
| | Kelp | 2 | 181.7 | $y=0.0015x^{2.6745}$ | 0.92 | 70 |

Data analysis: Kelp consumption rates were examined across all regions, but analysis of habitat effects were restricted to the north, southeast and southwest as only these regions exhibit both habitats. There was no change in area ($0.0 \pm 0.0 \text{ mm}^2$) of the kelp pieces in cages (controls) or those elevated off the substratum in the canopy (where they were exposed only to herbivorous fishes), therefore these treatments were not included in the overall analysis. Thus, a 2-way ANOVA was used, with the factors ‘Treatment’ (fixed, 2 levels: drift,

attached) and ‘Habitat’ (fixed, 2 levels: kelp, barrens) and loss of biomass (wet weight/day) as the response variable.

Behavioural response of sea urchins to drift-kelp availability

Experimental design: The change in activity of *H. erythrogramma* associated with the presence / absence of drift-kelp was monitored using time-lapse photography. The experiment was implemented as a before-after control-impact (BACI) design, whereby urchin movement within a total of eight plots of $\sim 0.6 \text{ m}^2$ on barrens habitat (Fig. 1, site 2) was monitored before and after the addition of drift-kelp to treatment plots ($n = 4$) alongside control plots ($n = 4$) to which no drift-kelp was added. The experiment was conducted during October 2014, with the BACI design involving an initial 4 hours of tracking before the drift-kelp treatment was added and then a subsequent 4 hours of tracking following the application of kelp. The treatment involved gently placing drift-kelp against all sea urchins within the field of view of the camera and within a 0.5 m buffer zone surrounding the camera field of view. Upon detection of the drift-kelp (a fresh cut lateral of *E. radiata*) urchins invariably extended their tube feet and seized the piece, which typically took on the order of 10 seconds. The images were analysed as described in the time-lapse experiment above, but only the speed of the urchins was used for analysis as displacement correlated highly with speed ($R^2 = 0.76$, $F_{1,27} = 87.45$, $p < 0.001$, transformation: $Y^{0.3}$) and thus was no more informative.

Data analysis: The experiment consisted of the two factors ‘Treatment’ (control and drift-kelp treatment group) and ‘Period’ (before and after addition of drift-kelp) with urchin speed (cm min^{-1}) as the response variable and was analysed using a 2-way nested ANOVA with the factors ‘Treatment’ (fixed, 2 levels: treatment and control), ‘Period’ (fixed, 2 levels: before and after) and ‘Plot’ (nested within Treatment by Period). Multiple comparisons (Tukey’s HSD) were conducted using the means of each plot (the number of urchins within each plot was similar, ranging between 8 and 13).

Results

Directional movement of urchins towards kelp

In 10 out of 11 instances, individual sea urchins moved directly towards the kelp pieces within the first few hours of the trials (Fig. 4). The exception was an urchin that did not move towards kelp, but moved out of the trial area entirely. This highly significant directional response (χ^2 -test: $F_1 = 7.36$, $p = 0.007$) demonstrates that *H. erythrogramma* can sense the kelp *E. radiata* and will actively move towards it. In 7 of the 10 successful trials, graze marks on the kelp pieces were observed when the urchin was removed from the kelp post trial.

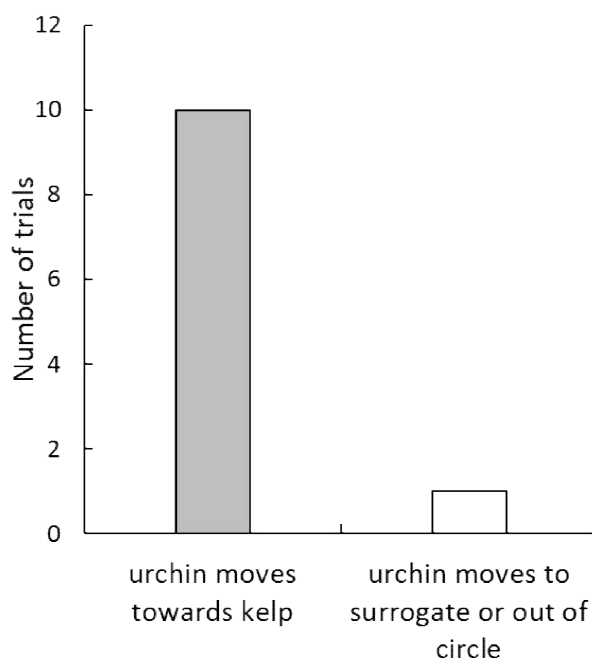


Figure 4. Frequency of trials resulting in sea urchins moving straight to kelp (grey bar) compared to the frequency of trials whereby sea urchins moved out of the circle (open bar). Note that none of the urchins moved to the surrogate.

Movement rates of urchins in different regions and habitats

Time-lapse monitoring revealed significant differences between ‘regions’ in the average speed and net displacement of sea urchins on barrens habitat (the barrens habitat was present across all regions). Urchins in the northern region of PPB showed the highest average speeds and net displacements, followed by those from the southeast and then the west (Table 1b,

‘Region comparison’, Fig. 5a). There were also significant differences in both movement parameters among plots within the same region, indicating that these characteristics vary on a spatial scale of 10s of meters.

Time-lapse monitoring also revealed a significant effect of habitat on sea urchin movement, with urchins showing significantly greater movement on barrens than in kelp bed habitat (Table 1c, ‘Habitat comparison’, Fig. 5b) for both speed and displacement. Furthermore, this effect was consistent across the north and southeast regions. Again there were significant differences in speed and displacement among plots within the same habitat and region (Table 1).

Kelp consumption rates in different habitats

On urchin barrens both attached kelp and drift-kelp were heavily grazed, with 67% of the ‘drift’ kelp pieces consumed entirely. Residual amounts of the kelp pieces were found in the jaws of the clothes pegs holding them (i.e. where grazers could not access), and in this circumstance it was deemed unlikely that the kelp piece was dislodged or torn away, and thus it was counted as being consumed. In contrast to the barrens habitat, in the kelp beds only the drift-kelp pieces were grazed (Fig 5b). When retrieving the ‘drift’ kelp pieces at the end of the assays, pieces were often either being grazed on by urchins at the time of collection or attached to urchins as ‘decoration’, therefore we concluded that tissue loss of the kelp pieces was due to the urchin grazing and not because of mechanical abrasion. This clear habitat effect was consistent in all regions and was independent of urchin density, occurring regardless of whether urchin biomass was higher on barrens or within kelp beds (note that in the north urchin biomass was higher in kelp beds than on barrens, whereas in southeast and southwest this pattern was reversed; Fig. 6a, Table 2). The ‘habitat effect’ of urchins grazing more on drift and attached kelp on the barrens than in the kelp beds was most clearly evident when considering the consumption rates of individual urchins (Fig. 6c). Results of the 2-way ANOVA (wet weight/day; pooling across region; transformation: $Y^{0.3}$) show that consumption of kelp was significantly different between treatments (higher consumption of drift-kelp than attached kelp; $F_{1,8} = 7.75, p < 0.05$) and habitats (more grazing on kelp pieces on the barrens than in the kelp habitat; $F_{1,8} = 26.4, p < 0.001$), and there was no habitat by

treatment interaction ($F_{1,8} = 2.81$, $p = 0.13$). Attached kelp within kelp beds was grazed significantly less than any other treatment or habitat (Tukey's HSD, $p < 0.05$, Fig. 6a).

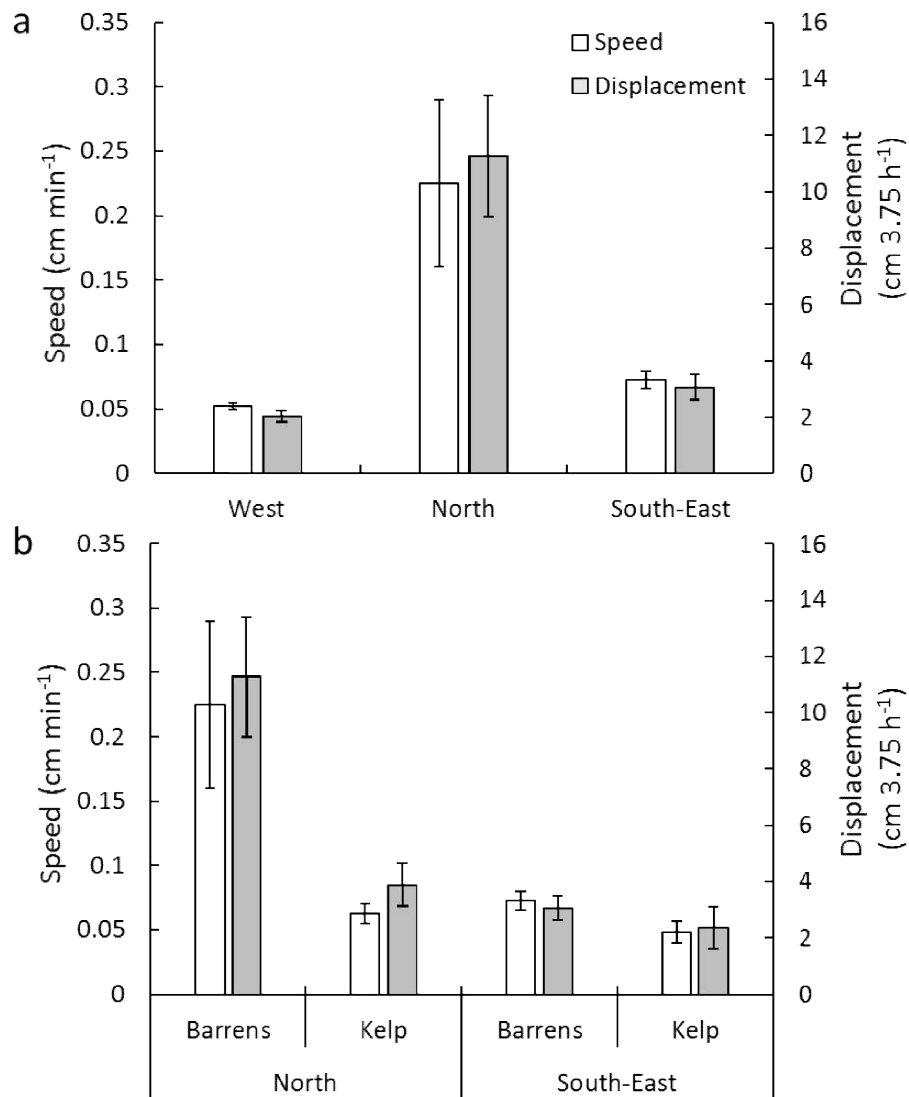


Figure 5. Comparison of sea urchin movement speed (cm min^{-1}) and displacement (cm 3.75 h^{-1}) as measured by time-lapse photography across (a) 3 regions of PPB (barrens habitat only), and (b) kelp bed vs urchin barrens habitats (both habitats were only present in 2 of 3 regions in PPB). Shown are the averages of each urchin per time-lapse sequence ($n = 6$, \pm SE).

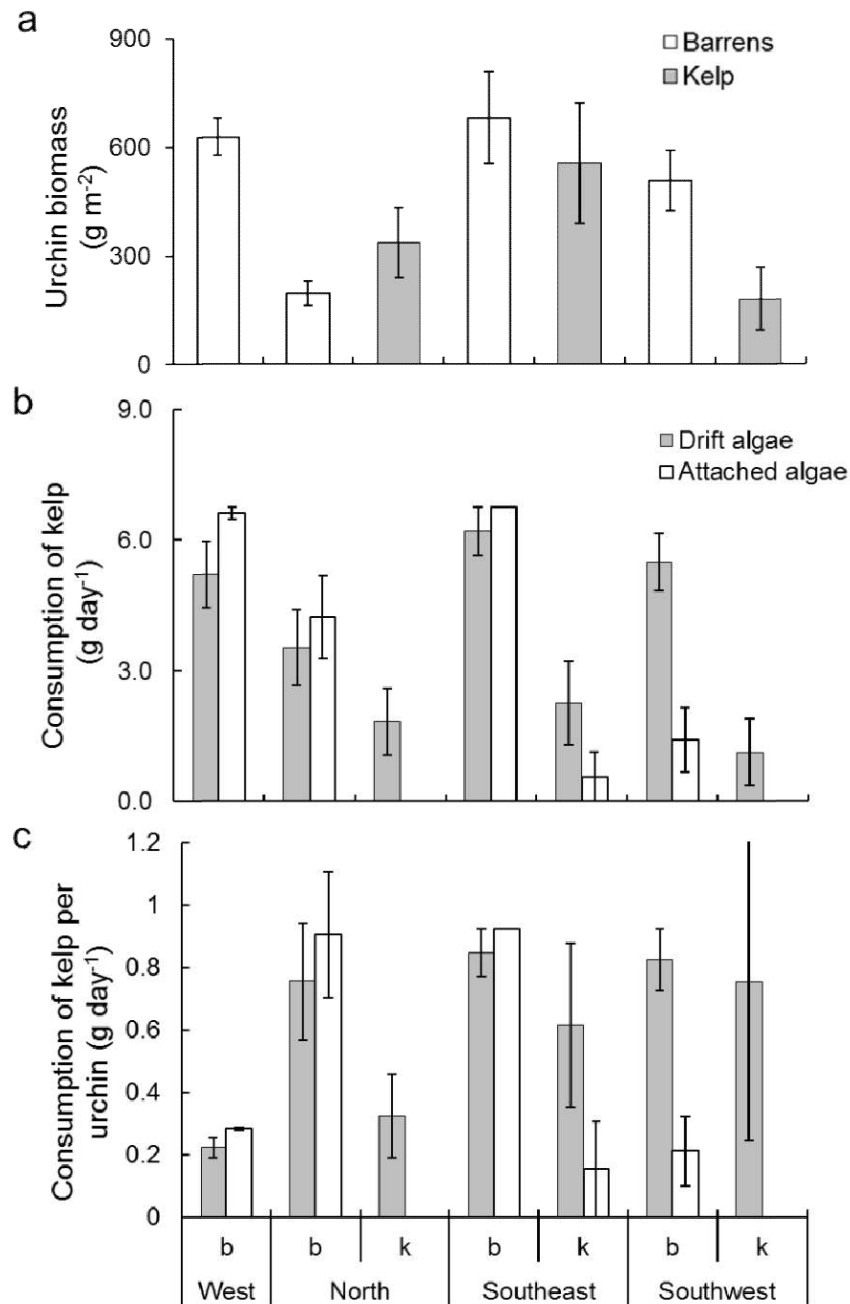


Figure 6. (a) Sea urchin biomass across 4 regions (west, north, southeast and southwest) of PPB; open bars display the barrens habitat and grey bars the kelp habitat ($n = 25$, \pm SE). (b) Consumption rates of kelp (g wet weight/day) after 3 days for different regions and habitats (b = barrens, k = kelp) and (c) consumption rates of kelp per urchins ($\text{g wet weight day}^{-1} \text{urchin}^{-1}$) after 3 days for different regions and habitats. Open bars show the grazing of kelp pieces mimicking attached kelp thalli and grey bars of kelp pieces mimicking drift-kelp ($n = 12$, \pm SE).

Behavioural response of sea urchins to drift-kelp availability

The speed of the control and treatment groups before addition of drift-kelp were similar, whereas the speed of the treatment group after drift-kelp addition was significantly lower than the other periods / treatments (Fig. 7). The movement of urchins receiving drift-kelp was noticeably less after drift was added (Fig. 7).

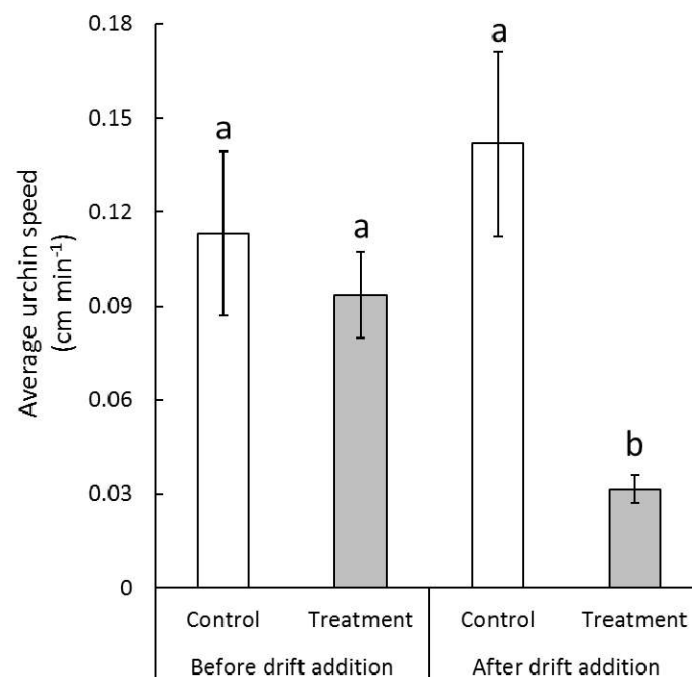


Figure 7. Change in speed (cm min^{-1}) of sea urchins before and after the controlled addition of drift-kelp. Bars left-of-centre show the speed of the control group (open bar) and treatment group (grey bar) without drift-kelp, while bars right-of-centre show mean urchin speed in the control (no added drift kelp) and treatment groups in the period after addition of drift-kelp to the treatment plots ($n = 4$, \pm SE). ANOVA (transformation Y^1) revealed a significant treatment by period interaction ($F_{1,12} = 14.2$, $p < 0.01$), and significant variation among plots within the same ‘treatment’ and ‘period’ ($F_{12,103} = 2.45$, $p < 0.01$). Bars sharing the same letters are not significantly different (Tukey’s HSD, $\alpha = 0.05$).

Discussion

The top-down control of consumption, either of herbivores feeding on primary producers or predators on their respective prey, can have a large influence on prey biomass and thus on overall ecosystem structure (Rosenzweig & MacArthur 1963, Crawley 1983, Hanski & Korpimäki 1995, Guarnieri et al. 2014). For Australian temperate rocky reefs, *H. erythrogramma* is an important herbivore capable of reconfiguring the structure of the reef ecosystem it inhabits (Valentine & Johnson 2005a, Wright et al. 2005, Ling et al. 2010). The current research provides clear experimental data indicating that when adequate amounts of drift-kelp are available to urchins, kelp beds will be resilient to grazing. The experiments revealed the nature of urchin grazing, and the underlying factors determining this behaviour: (1.) *H. erythrogramma* can chemically sense *E. radiata* and actively moves towards it promoting the formation of feeding aggregations observed for this species; (2.) *H. erythrogramma* is less vagile and consumes less attached kelp in kelp beds than when individual kelps are temporarily present on barrens; and (3.) *H. erythrogramma* displays behavioural plasticity, with switches in feeding behaviour triggered by the supply of allochthonous food in the form of drift-kelp.

Directional movement of urchins towards kelp

The experiments showed that *H. erythrogramma* can sense *E. radiata* as a food source and actively moves towards and consume it. Few studies have distinguished between whether urchins can sense algae or whether they move essentially randomly until they encounter food. Rodriguez and Farina (2001) manipulated drift-algae and observed the urchin *Tetrapygus niger* to form aggregations around drift-algae, suggesting that this species can also sense algal food sources and direct movement towards it. For other species like *Strongylocentrotus droebachiensis* (Harding & Scheibling 2015) and *Centrostephanus rodgersii* (Flukes et al. 2012), experiments have indicated no directed movement towards algae. Thus, it would appear that the ability of sea urchins to sense food is species-specific.

Movement rates of urchins in different regions and habitats

Time-lapse monitoring revealed high variability in movement rates among individual urchins within each plot. Nevertheless, overall patterns could be observed; regionally, urchins were on average more active (in terms of both speed and net displacement) in the north of PPB, followed by the southeast region, and moved least in the west. An explanation for this difference in movement could not be identified, although all three regions show unique environmental features. A relationship with density was examined since urchin densities are extremely high in the west, but the correlation was low and not significant ($R^2 = 0.02$, $F_{1,28} = 1.55$, $p = 0.22$, transformation: $Y^{0.3}$), even though other studies have reported movement dependent on density (Lauzon-Guay & Scheibling 2007).

In accordance with other studies (Harrold & Reed 1985, Konar 2001, Dumont et al. 2007), a key result of the time-lapse monitoring was that urchins do not move as much in kelp habitats as on barrens habitat. Urchins on barrens habitat moved more rapidly and covered greater distances than their counterparts in nearby kelp beds, which suggests that they actively forage and seek food. The simplest interpretation is that food in kelp beds is more abundant, therefore urchins do not have to forage as far to access it. In addition, urchins may move less in kelp habitat in response to the whip-lash effect of the kelp sweeping the substratum with their laminae (Konar 2000), or because increased presence of predators (e.g. lobsters, large crabs, or fish) leads to urchins seeking and remaining for longer periods in crevices (Ling & Johnson 2012). Arguably, the most accepted hypotheses are that reduced movement of urchins in kelp arises because food is in closer proximity in kelp beds (Mattison et al. 1977, Russo 1979), and / or that more detached drift-algae is available in kelp beds so that urchins can remain stationary within crevices and catch drift with their tube feet as it moves around on the benthos (Harrold & Reed 1985, Konar & Estes 2003).

Kelp consumption rates in different habitats

The clear difference between consumption rates on attached algae in kelp beds and on urchin barrens supports the notion that in kelp beds, providing that drift-algae is available, urchins feed on drift without consuming attached algae, while on barrens habitat with diminished food availability they feed on any suitable algae they encounter. This finding is consistent with that of Vanderklift et al. (2009) who conducted a similar experiment in kelp beds in

Western Australia and found that *H. erythrogramma* primarily consumed drift algae in kelp beds. It is possible that the higher grazing rates on barrens might reflect higher urchin population densities (or biomass density) in this habitat, but this was not the case in the current study since the results were independent of the biomass and density of sea urchins. When calculating the consumption per urchin it was revealed that urchins on barrens habitat consumed more kelp pieces than those in kelp, thus food availability within habitat types emerge as a key factor in understanding the spectrum of urchin feeding behaviour.

Behavioural response of sea urchins to drift-kelp availability

The commonly held notion that decreased feeding on attached kelp in kelp habitat is dependent on the availability of drift (Harrold & Reed 1985, Vanderklift & Kendrick 2005) was supported by the field experiment conducted here. Correspondingly, Mattison et al. (1977) observed in California that *Strongylocentrotus franciscanus* feeding rates on drift-algae decreased with increasing distance from kelp beds, whereas total movement showed the inverse and increased with increasing distance from kelp beds. These authors postulated that decline in drift-kelp was the reason for increased movement, because urchins would actively have to forage further to obtain food. Since then, various observational studies have explored the relationship between movement and drift-algal availability (Dean et al. 1984, Day & Branch 2002), however studies manipulating drift-kelp are rare (but see Vanderklift & Wernberg 2008). The response observed to manipulation of drift-kelp availability clearly strengthens the notion that drift-kelp is a major determinate of foraging dynamics and thus a key control of the propensity for overgrazing.

Conclusions

The dynamic foraging traits of *H. erythrogramma* evident in the experiments here concord with patterns of urchin grazing observed across Australia (Vanderklift & Kendrick 2005, Wright et al. 2005, Vanderklift & Wernberg 2008, Ling et al. 2010). That is, experimental results of the current study support observations that *H. erythrogramma* only destructively overgrazes attached kelp on sheltered inshore reefs where drift-kelp supply is low, whereas the same densities of urchins are observed to have nil influence on the biomass of attached

kelps on exposed offshore reefs with higher supply of drift-kelp (Vanderklift & Kendrick 2005, Vanderklift & Wernberg 2008). The experiments in the current study are consistent with the notion that reduced foraging movements are associated with ready availability of drift within kelp beds, and additionally that destructive grazing can be triggered by a decline in the availability of allochthonous drift-kelp food. It is concluded that the presence of sufficient drift-kelp, either generated from the natal kelp bed (autochthonous) or from distant allochthonous sources (Vanderklift & Wernberg 2008), suppresses urchin movement and the propensity for destructive grazing and increases the resilience of kelp beds to overgrazing. Conversely, as kelp beds undergo gradual decline (Steneck et al. 2002), lower availability of drift-kelp will create a positive feedback loop accelerating the process of overgrazing and ultimately kelp bed loss.

Chapter 6: General discussion

This thesis aimed to determine the underlying dynamics of shifts of rocky reefs systems from kelp beds to sea urchin barrens habitat under the influence of urban stressors. The study was conducted on reefs in Port Phillip Bay (PPB) in southeastern Australia, which experiences a strong gradient in urbanisation (Harris et al. 1996). Across this gradient rocky reef communities occur in different states and at different transitional stages. Southern regions display diverse kelp beds, whereas the northern and in particular western regions are dominated by sea urchin barrens. This spatially- and temporally-dynamic system provides an excellent opportunity to test phase-shift dynamics of kelp beds under multiple human-stressors. Furthermore, given anecdotal evidence of a decline in kelp beds and expansion of urchin barrens throughout the Bay (Jung et al. 2011), the study also provides an opportunity to identify potential management actions to conserve native kelp bed ecosystems. This study specifically aimed to provide in-depth information regarding the influence of urban stressors of sedimentation and nutrification on the potential for phase-shift on rocky reefs. Additionally, causative agents driving the collapse of kelp beds and critical tipping-points involved in this transition were explored. This was achieved through a suite of novel field experiments. The experiments are *in situ* therefore they capture the dynamics better than laboratory experiments, the size is workable on SCUBA, and a lot of different factors can be measured and monitored and the disturbance to the organisms worked with can be minimised. Even though small in scale, the rates of algal recovery observed in the absence of urchin grazing are informative, and there are many larger scale experiments which show that altering urchin densities scales well from small to large scales (Ling et al. 2015). The experiments presented in this thesis extend existing knowledge about reef ecosystem collapse and kelp bed / urchin barrens dynamics (reviewed by Filbee-Dexter & Scheibling 2014, Ling et al. 2015), and provide critical tests of top-down vs bottom-up control of this phase-shift.

The effects of multiple stressors on macroalgal dynamics

Chapters 2 and 3 of this thesis examined changes in the composition of macroalgal communities and areal cover of macroalgae when subjected to elevated nutrients and

sediments, in combination with sea urchin herbivory. In line with existing literature (Lawrence J. 1975, Chapman 1981, Wright et al. 2005, Ling 2008, Guerry et al. 2009), it was found that the top-down pressure of grazing, in this case by the urchin *Heliocidaris erythrogramma*, controls both algal abundance and the composition of the algal community. This was revealed in an experiment (Chapter 2) conducted on urchin barrens at sites spanning the western, northern and southeast regions of PPB representing high, medium and low nutrient / urbanisation levels respectively. Across these regions, removal of sea urchins led to variable responses in the rates and patterns of recovery for algal communities, with recovery rates positively correlated with increasing nutrient inputs (due to the treatment plant in the west and the Yarra River in the north) clearly indicating the role of bottom-up forcing. While bottom-up effects were evident, the top-down effect of sea urchin grazing demonstrated an overwhelming negative effect on macroalgal cover. The western region showed the highest productivity (and nutrient levels), but also was most affected by grazers (and demonstrated the most extensive urchin barrens). This is contrary to several other studies showing that grazers have less effect on the abundance of algae in high-productivity areas (reviewed by Worm et al. 2002, Burkpile & Hay 2006). In PPB the urchin abundance is positively correlated with nutrient input, with the highest abundance of urchins in the west, followed by the north and then the southeast.

Community composition differed in the presence and absence of sea urchins in all but the most highly nutrified western region, where the species proliferating in the absence of sea urchins were the same as those found in the presence of urchins, predominantly *Ulva sp.* and *Undaria pinnatifida*. Both are fast growing, annual algae species that show an affinity to high nutrient areas (Lobban & Harrison 1994, Campbell 1999). *U. pinnatifida* is an introduced annual canopy-forming algae (Campbell & Burridge 1998), and represents the only canopy-forming algae in the north west of the Bay. This region is dominated by expansive urchin barrens that can span thousands of square meters (pers. obs.), thus over most of this region mature sporophytes of *E. radiata* are too far away to provide a meaningful density of gametophytes for fertilisation, which prevents recovery of macroalgal communities even when urchin grazing is eliminated. The possible reason *U. pinnatifida* successfully inhabits this region and *E. radiata* is absent, is that *U. pinnatifida* grows fast enough to outstrip the urchins' capacity to destructively overgraze. Once *U. pinnatifida* established on reefs in this region, sufficient individuals appear to outgrow the grazing pressure and subsequently

provide a local spore supply. Valentine and Johnson (2005b) found the same pattern in Tasmania where *U. pinnatifida* successfully colonised urchin barrens devoid of native kelp.

In Chapter 3, the correlative pattern between nutrient input, sea urchin abundance and algal community composition was further examined in a manipulative experiment. In the region with the lowest nutrient input and high percentage of diverse kelp beds the effects of (1) bottom-up nutrient forcing, (2) top-down grazing control, and (3) the urban stressor of sedimentation were examined to identify the role of these putative drivers of reef community composition. In an intact kelp bed in the southeast of PPB, nutrient and sediment levels were enhanced in order to identify their effect on kelp beds. These drivers were examined because of the historic association of sediment and nutrient run off with human population growth and increasing coastal urbanisation (Nixon 1995, Carpenter et al. 1998, Cloern 2001, Airoidi 2003, Nearing et al. 2004, Russell et al. 2009), and anecdotal evidence of an increase in urchin abundance in PPB (Jung et al. 2011).

Nutrients had a positive effect on algal cover (predominantly the kelp *Ecklonia radiata*), reiterating the observed pattern for annual species in Chapter 2. In contrast to other studies (Pfister & Van Alstyne 2003, Kraufvelin et al. 2010), the experiment identified that the cover of adult kelp was increased with an increase in nutrient load. This finding is also inconsistent with previous research that identified that turf-forming algae can benefit from nutrient addition at the expense of canopy-forming kelp (Worm et al. 1999, Gorgula & Connell 2004). Most studies exploring this relationship between turf and canopy-forming algae assessed ecosystems where canopy-forming algae were absent (Worm et al. 1999, Benedetti-Cecchi et al. 2001, Gorgula & Connell 2004, Russell & Connell 2005) and found that once kelp is lost, available space was occupied by turf-forming algae, which in turn prevents kelp recruitment (Valentine & Johnson 2005a, Irving et al. 2009). Our experiment however, revealed that turf-forming algae was negatively influenced by the enhancement of nutrient, which we interpret as an indirect consequence of nutrient enhancement; enhanced nutrient had a direct positive effect on the cover of *E. radiata*, which in turn reduced turf cover through the sweeping action of kelp thalli on the substratum and increased shading (Kennelly 1989, Connell 2005, Russell 2007, Falkenberg et al. 2015).

The experiment described in Chapter 3 is one of the few studies conducted in an intact kelp bed (but see Bokn et al. 2003) and not on isolated kelp individuals (Pfister & Van Alstyne 2003) or on juvenile kelps (Amsler & Neushul 1990, Van Alstyne & Pelletreau 2000). The

experiment demonstrates the resilience of intact kelp beds to a transition towards turf-forming algae under increasing nutrient regimes providing that sea urchin densities are less than the critical threshold in herbivory that limits kelp development. If the critical threshold in urchin density / density biomass is reached the positive effect of nutrients is ultimately surpassed by the grazing pressure of the sea urchins.

As shown for other systems (Lawrence J. 1975, Hillebrand 2003, Atalah & Crowe 2010, Smith et al. 2010, Guarnieri et al. 2014, Ling et al. 2015), the algal community across vast reef areas of PPB is controlled by the top-down force of grazing (Chapters 2&3). It is revealed here that the main driver of loss of kelp and other canopy-forming seaweeds in PPB is the sea urchin *H. erythrogramma* and therefore it was vital to examine the behavioural dynamics of grazing by this key herbivore. Additionally, gaining knowledge about the thresholds of sea urchin densities and biomass within kelp beds and on urchin barrens can help inform natural resource managers to understand the likelihood of transition between the two states and potential options for preventing kelp bed collapse, and/or remediating it where it has been lost.

Effects of urchin abundance and dynamic feeding behaviour

Chapter 4 and 5 describe experiments exploring the destructive overgrazing of the sea urchin *H. erythrogramma* in PPB. First, we attempted to identify tipping-points and estimate the presence and magnitude of hysteresis in the phase-shift between kelp beds and urchin barrens by using a manipulative field experiment, and second, the plasticity of urchin foraging behaviour causing destructive overgrazing was examined.

The experiment described in Chapter 4 aimed to determine the population and biomass density necessary to cause collapse of kelp beds through overgrazing, and conversely, the population and biomass density to which urchins need to be reduced to enable kelp recovery on urchin barrens. Furthermore, nutrients were enhanced to assess whether tipping points were sensitive to nutrient regimes. The results are consistent with the notion that kelp bed and barrens habitat represent alternative stable states (Boesch 1974, Johnson & Mann 1988, Ling et al. 2009a, Filbee-Dexter & Scheibling 2014, Ling et al. 2015) and for PPB we estimate the tipping-points for the ‘backwards’ and ‘forwards’ shifts are between 4 and 8 urchins m^{-2} (213 and 427 g m^{-2} respectively). This is one of the first experiments to explore tipping-points

for kelp bed collapse and recovery simultaneously. Thus far, studies detailing multiple tipping-points and the hysteresis between them either assess either overgrazing or recovery (but not both simultaneously) and rarely consider gradients in urchin abundance (but see Andrew & Underwood 1993, and Hill et al. 2003, reviewed by Filbee-Dexter & Scheibling 2014, and Ling et al. 2015). Typically in these studies, researchers attempt to remove all sea urchins from experimental plots, however a few studies (Andrew & Underwood 1993, Benedetti-Cecchi et al. 1998, Hill et al. 2003) reduced urchin densities to half or one or two thirds of natural occurring densities, but none of these experiments incrementally removed urchin as was conducted for Chapter 4.

The experiment additionally showed greater recruitment of kelp in the presence of adult sporophytes when urchin abundance was low. This suggests that kelp recovery can be supported by adult kelp individuals in the vicinity. In contrast to other studies which were conducted in different seasons and within existing kelp beds (Carnell & Keough 2014, Flukes et al. 2014), our results showed that newly recruited kelp sporophytes would appear more quickly near adult conspecifics, potentially due to the scouring effect of adult kelp sporophytes sweeping the substratum in surge (Kennelly 1989, Russell 2007) and keeping it clear of any understorey and turf-forming algae. Proximity to spore sources can be ruled out as a possible reason for this pattern as all experimental plots were supplied with kelp spores by hanging adult individuals in onion bags over them.

The enhancement of nutrients did not alter tipping points defined by urchin abundance and biomass densities. Moreover, the nutrient enhancement had no effect on kelp percentage cover or algal community composition in northern PPB where the experiment was conducted. This is contrary to our finding in the southeast (Chapter 3), where enhancement of nutrients using an identical treatment resulted in a positive effect on kelp cover. A possible explanation is that the growth of kelp in the southeast is nutrient limited, whereas in the north the kelp receives sufficient nutrients due to the vicinity of the metropolitan area and major water outlets (e.g. Yarra river) bringing nutrients into this region.

Chapter 5 describes the results of a set of experiments detailing the behavioural plasticity of *H. erythrogramma* related to sea urchin overgrazing. The experiments of the previous chapters indicate the destructive overgrazing role of *H. erythrogramma* (Wright et al. 2005, Ling et al. 2010). However, this species can also inhabit kelp bed habitat benignly without causing overgrazing, even at high densities that elsewhere lead to destructive overgrazing

(Vanderklift & Kendrick 2004). Interestingly, *H. erythrogramma* is found to overgraze kelp beds in New South Wales (Wright et al. 2005), Tasmania (Johnson et al. 2004, Ling et al. 2010), and Victoria (this study), but not in Western Australia (Connell & Irving 2008) or South Australia. This raises the question as to why this species is destructive in eastern Australia but ecologically benign in another at identical densities, and suggests a complex grazing behaviour that is not simply a function of density. The underlying dynamics of alternative behavioural modes were considered to examine the mechanisms leading to changes in feeding behaviour that affect the probability of urchin overgrazing. The experiments tested and confirmed the assumption that the availability of drift-kelp plays a vital role in determining the foraging behaviour of the sea urchins (Harrold & Reed 1985, Vanderklift & Kendrick 2005, Vanderklift et al. 2009). Within intact kelp patches, when drift-kelp is available urchins do not actively forage on attached kelp, but instead remain sedentary and feed on drift-kelp. They only actively move towards attached kelp and graze it when the supply of allochthonous drift-kelp is depleted. It therefore appears possible to maintain urchins at high densities in a benign feeding mode providing that drift-kelp is supplied to keep destructive feeding mode at bay. The bottom-up supply of drift-kelp therefore appears a critical component on the 'productivity' of any given reef, and thus changes to productivity in this respect will have a large bearing on the phase-shift potential of particular reefs.

Synthesis

The results from this thesis show that the algal communities on rocky reefs within PPB are largely controlled by the top-down driver of urchin grazing, and in turn urchin feeding behaviour is determined by the bottom-up effect of the nature of food availability. The availability of abundant drift-kelp changes urchin foraging behaviour from active foraging on attached sporophytes to feeding in a sedentary state. Notably, the top-down control was not influenced by attempting to change the resilience of existing kelp beds with nutrient or sediment enhancement. In combination these stressors revealed an antagonistic effect between herbivory (which depletes kelp biomass) and nutrient enhancement (which stimulated growth of attached kelp). The phase-shift dynamics presented here are similar to top-down triggering of regime-shift observed in other ecosystems. In marine environments a similar shift can occur on coral reefs, which transition from coral to algal dominated

communities when herbivory is reduced, e.g. as a result of intensive fishing of herbivorous fishes or mass mortality of the herbivorous sea urchin *Diadema antillarum* (Carpenter 1990, Nyström et al. 2000). Interestingly, at least at some sites, the transition from a coral- to algal-dominated system can also be driven by eutrophication without any significant change in levels of herbivory (Arias-González et al. 2017). In freshwater habitats, the shift from clear water lakes with high vegetation to lakes with turbid water and high amounts of phytoplankton offers another example of phase shift (Scheffer et al. 1993). Although the main driver in this shift is also thought to be eutrophication, grazing by *Daphnia* spp. plays a vital role in restoring lakes to a pristine state once their predators are reduced and nutrient levels are moderated (Meijer et al. 1994). In terrestrial environments one of the most frequently suggested examples of continuous phase shift (i.e. with no hysteresis) is the transition from woodland habitat to grassland wherein a reduction of grazing by herbivores leads to reestablishment of woodlands (Dublin et al. 1990). These examples demonstrate that phase-shifts occur globally and across many differing habitat types, and herbivory often plays a vital role influencing how such habitats will recover or degrade. In marine seaweed-based systems herbivory by urchins can lead to a degraded state largely devoid of large macroalgae, whereas in other habitats it helps the recovery process. While phase shifts may be readily recognised, it is often less clear whether they represent discontinuous shifts with hysteresis and, by definition, multiple stable states, or continuous phase shifts with no hysteresis.

Utilising the information gathered within this thesis, it is possible to estimate the likelihood that kelp beds will undergo a phase-shift to urchin barrens and whether existing urchin barrens can successfully be recolonised by kelp. This is achievable by assessing urchin biomass density, as discussed in chapter 4, which enables management strategies to be developed to protect existing kelp beds or to improve the recovery of kelp beds post phase-shift. The results demonstrate how sea urchin grazing shapes rocky reef ecosystems in PPB and that neither elevated nutrient nor sedimentation levels, within the bounds manipulated here, alter the dominance of the top-down force of the urchins. In a management context, findings from this thesis suggest that the overabundance of urchins is the preeminent reason causing the collapse of kelp beds in PPB.

Urchin barrens represent an alternative stable state and therefore possess self-stabilising feedbacks, which impede kelp bed recovery (Fig. 1, ‘urchin barrens’). This means that the density of urchins to maintain an urchin barren once it is formed is less than at the initial

tipping point in the transition from kelp bed towards a barrens state. Consequently to promote kelp recovery, urchins have to be removed from the system, e.g. by culling, harvesting and/or natural predation. Most of the populations of urchins in PPB appear self-recruiting and show little connectivity between each other, due to their short several-day planktonic phase. *H. erythrogramma* displays a lecithotrophic development (Williams & Anderson 1975) and settles within 5 to 6 days after fertilisation. It logically follows that, particularly in an enclosed embayment, such as PPB, where oscillatory tidal forcing is the predominant feature of circulation, that net transport away from the natal reef might be quite small. This implies that urchin biomass reduction can be achieved locally by removing urchins from reefs without large risk of high recruitment again in the next summer season. This thesis provides clear guidelines on how much the urchin biomass needs to be reduced to ensure recovery of kelp ($\leq 213 \text{ g m}^{-2}$). Most other studies assessing kelp recovery have removed urchins entirely (e.g. Villouta et al. 2001, Ling 2008, Ortega-Borges et al. 2009, Ling et al. 2010, Bonaviri et al. 2011, Newcombe et al. 2012). While such removals have led to successful macroalgal recovery, the approach of testing specific thresholds in urchin densities that allow kelp bed recovery can enable managers to evaluate the severity of the degradation of the reefs and prioritise where urchin removals are likely to achieve greatest kelp bed recovery.

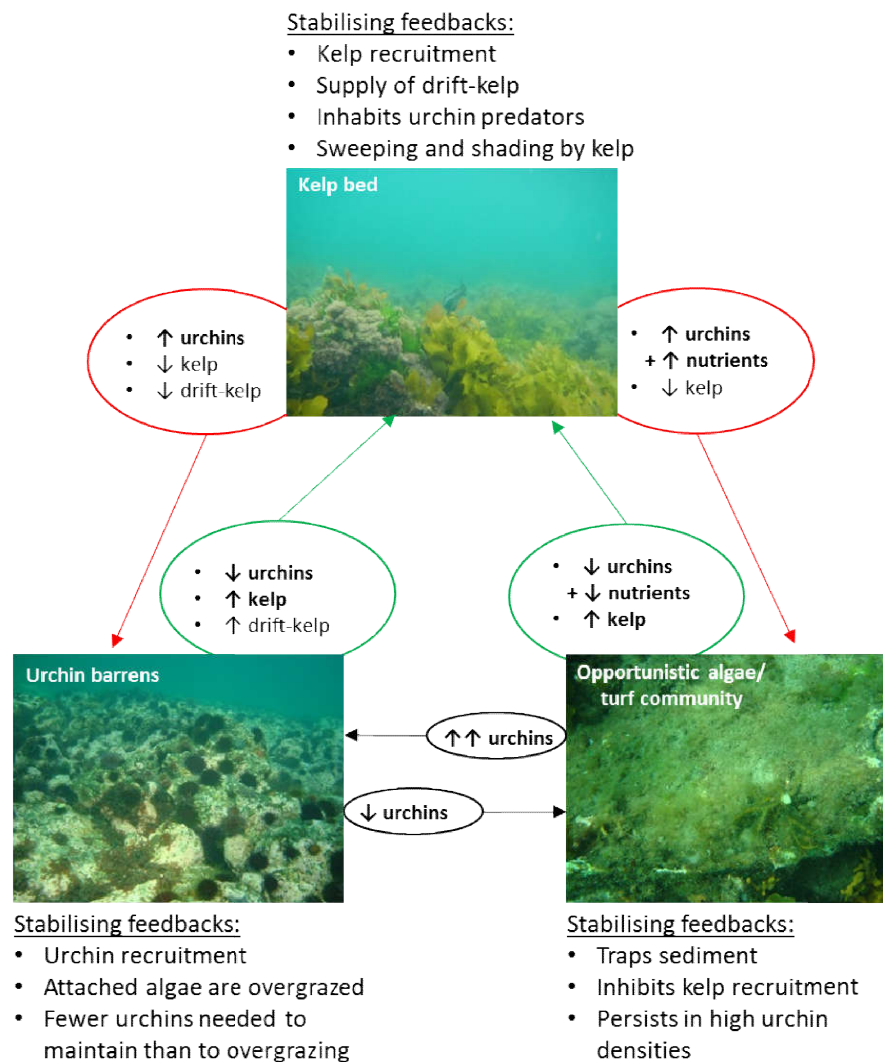


Figure 1. Conceptual diagram representing the different environmental states of PPB. The pictures show the 3 different potential states, two of which are assessed in this thesis, while the third, ‘opportunistic algae / turf community’ was not specifically assessed here, has been examined by other researchers (e.g. Benedetti-Cecchi et al. 2001, Gorgula & Connell 2004, Russell & Connell 2005). Presented are the states and important positive feedback mechanisms (‘Stabilising feedbacks’) together with the main impacts enabling change from one state to another. The green circles enclose impacts favouring a shift to kelp beds whereas red circles identify impacts leading to a collapse of kelp and shift to states devoid of kelp. The transition between the ‘Urchin barrens’ and ‘Opportunistic algae / turf community’ is mainly caused by urchin abundance (black arrows), but can also coexist with similar urchin densities except if they are extremely high (e.g. in the west of PPB with 39 urchin m⁻²). The arrows leading from the circles display the direction of shift and the arrows in front of the variables represent whether they have to increase (↑) or decrease (↓) to cause a transition. Variables in bold emphasise greater influence over the shift.

Unfortunately, by solely removing urchins there remains the risk that instead of kelp, turf algae or other fast growing opportunistic algae will fill the available space (Fig 1, ‘opportunistic algae / turf community’). Other research has shown that under high nutrient regimes, turf-forming algae proliferate (Gorgula & Connell 2004, Russell et al. 2009) and the experiment detailed in Chapter 2 of this thesis demonstrated that reefs are quickly occupied by annual species, before perennial canopy-formers recruit. In this experiment, fast growing annual algae species proliferated, particularly in the west and north of PPB where turf-forming algae dominated with most of the urchin barrens in these regions showing moderate to high development of turf. To avoid reef systems switching from barrens to a community dominated by turf and opportunistic annual algae, it is vital to ensure local supply of kelp spores. In regions like the west and north of PPB that lack existing kelp beds, local spore supply is clearly insufficient to generate gametophyte densities leading to successful fertilisation and development of sporophytes. Transplantation of mature adult kelp sporophytes following urchin removals would likely represent an optimal intervention to restore kelps in this area as this not only provides a spore supply but the sweeping and shading of the adult kelp prevents turf and other understory algae from dominating and further inhibiting kelp development (Fig 1, green circles).

In remnant kelp beds, when urchins are below the threshold biomass density and drift algae is available, the risk of a transition to urchin barrens is low (Fig 1, ‘Kelp bed’). To protect the kelp beds, management strategies should include evaluation of the urchin biomass within kelp beds to be able to determine the level of risk. For example, if urchins occur in high numbers, i.e. a biomass density above the threshold assessed in Chapter 4, then it is at a high risk to be overgrazed (Fig 1, red circles). In this case intervention to remove urchins from within and on the edges of the bed would be a useful tool to stabilise the kelp bed. Furthermore, the size of a kelp bed can also give an indication of the risk of overgrazing. Smaller kelp beds imply less drift kelp supply, due to fewer kelp individuals, so that urchins are more likely start feeding on attached kelp, accelerating the shift to a barrens state.

The experiments contained within this thesis have demonstrated that the recovery of kelp beds once extensive urchin barrens have formed cannot be achieved easily, but is labour and time intensive. Taking a pre-emptive approach and preventing overgrazing of existing kelp beds in the first place would be the preferable option, but unfortunately kelp has already been lost from large areas of PPB previously supporting kelp beds, while other kelp-dominated

areas are presently at high risk of overgrazing and transition to barrens. The economic and social importance of PPB demands that action to halt further collapse of reefs in this important system is taken to ensure the ecological, recreational, and commercial value of this unique ecosystem is conserved. This highly urbanised embayment experiences low water exchange with the open ocean and thus a long flushing time (~ 12 months) and is relatively shallow with an average depth of 8 m and only 24 m at the deepest point (Harris et al. 1996). The Bay is heavily used recreationally and commercially (shipping, fishing, water sports) and its coastline is subject to industrial and agricultural activity. These environmental characteristics make PPB unique in south-eastern Australia, but can be used as a model system for other enclosed embayments and estuaries or reefs where ongoing urbanisation is a threatening process.

Management implications and future research

From a management perspective, removing sea urchins and providing spore supply of the desired kelp species is likely to be the most effective way – and arguably the only means – of restoring kelp beds. It will be important however to consider the timing of urchin removals to coincide with seasonal spores release of preferred algal species to minimise the risk that turf-forming and other opportunistic algae will occupy and hold space on the reef. Furthermore, to protect existing kelp beds the likelihood of overgrazing should be evaluated by assessing urchin biomass within the kelp bed so that proactive action can be taken before destructive grazing occurs. If natural predators of urchins were still present, one option could be to create marine protected areas which would lead to higher predation on urchins (e.g. Shears & Babcock 2002, Pederson & Johnson 2006). In PPB this is not an immediate option due to the current lack of natural predators relative to historical baselines (Jung et al. 2011), but in the longer term this may be possible if the implementation of marine protected areas is combined with managed translocation (Johnson et al. 2013).

Given the key role of urchins as the primary driver of community structure on reefs in PPB, it would be sensible that future research would consider the controls of urchin population abundance. A contributing factor for the high abundance of urchins in PPB is likely that it represents an ideal environment for sea urchins, due to the sheltered nature of its rocky reefs systems, with low wave exposure relative to the open coast. Combined with intense fishing of

species known to prey on sea urchins (such as sparids, Shears & Babcock 2002, and spiny lobsters, Ling & Johnson 2012), urchins are able to thrive within this system. Further research should explicitly assess factors influencing urchin population dynamics in the Bay.

Beyond factors influencing per capita effects on sea urchin populations, the importance of the availability of drift-kelp in suppressing destructive behaviour of urchins seems clear, but quantitative estimates of the amount of allochthonous food required to suppress overgrazing are still lacking. More detailed quantitative knowledge of specific amounts of urchin consumption and drift-kelp production on reefs would help to identify thresholds in the availability of drift material and the likelihood of phase-shift.

References

- Agatsuma Y, Endo H, Yoshida S, Ikemori C, Takeuchi Y, Fujishima H, Nakajima K, Sano M, Kanezaki N, Imai H, Yamamoto N, Kanahama H, Matsubara T, Takahashi S, Isogai T, Taniguchi K (2014) Enhancement of Saccharina kelp production by nutrient supply in the Sea of Japan off southwestern Hokkaido, Japan. *J Appl Phycol* 26:1845-1852
- Airolidi L (2003) The effects of sedimentation on rocky coast assemblages. *Oceanography and Marine Biology*, Vol 41 41:161-236
- Albert S, Udy J, Tibbetts I (2008) Responses of algal communities to gradients in herbivore biomass and water quality in Marovo Lagoon, Solomon Islands. *Coral Reefs* 27:73-82
- Amsler CD, Neushul M (1990) Nutrient stimulation of spore settlement in the kelps *Pterygophora californica* and *Macrocystis pyrifera*. *Marine Biology* 107:297-304
- Andrew NL (1993) Spatial heterogeneity, sea urchin grazing, and habitat structure on reefs in temperate Australia. *Ecology* 74:292-302
- Andrew NL, Underwood AJ (1993) Density dependent foraging in the sea urchin *Centrostephanus rodgersii* on shallow subtidal reefs in New South Wales, Australia. *Mar Ecol-Prog Ser* 99:89-98
- Annala M, Mykra H, Tolkkinen M, Kauppila T, Muotka T (2014) Are biological communities in naturally unproductive streams resistant to additional anthropogenic stressors? *Ecol Appl* 24:1887-1897
- Arias-González JE, Fung T, Seymour RM, Garza-Pérez JR, Acosta-González G, Bozec Y-M, Johnson CR (2017) A coral-algal phase shift in Mesoamerica not driven by changes in herbivorous fish abundance. *PloS one* 12:e0174855
- Atalah J, Crowe TP (2010) Combined effects of nutrient enrichment, sedimentation and grazer loss on rock pool assemblages. *Journal of Experimental Marine Biology and Ecology* 388:51-57
- Balata D, Piazzzi L, Cinelli F (2007) Increase of sedimentation in a subtidal system: Effects on the structure and diversity of macroalgal assemblages. *Journal of Experimental Marine Biology and Ecology* 351:73-82
- Bearham D, Vanderklift MA, Gunson JR (2013) Temperature and light explain spatial variation in growth and productivity of the kelp *Ecklonia radiata*. *Mar Ecol-Prog Ser* 476:59-70

- Beisner BE, Haydon DT, Cuddington K (2003) Alternative stable states in ecology. *Frontiers in Ecology and the Environment* 1:376-382
- Benedetti-Cecchi L, Bulleri F, Cinelli F (1998) Density dependent foraging of sea urchins in shallow subtidal reefs on the west coast of Italy (western Mediterranean). *Mar Ecol-Prog Ser* 163:203-211
- Benedetti-Cecchi L, Pannacciulli F, Bulleri F, Moschella PS, Airolidi L, Relini G, Cinelli F (2001) Predicting the consequences of anthropogenic disturbance: Large-scale effects of loss of canopy algae on rocky shores. *Mar Ecol-Prog Ser*:137-150
- Berelson WM, Heggie D, Longmore A, Kilgore T, Nicholson G, Skyring G (1998) Benthic nutrient recycling in Port Phillip Bay, Australia. *Estuar Coast Shelf Sci* 46:917-934
- Berglund J, Mattila J, Ronnberg O, Heikkila J, Bonsdorff E (2003) Seasonal and inter-annual variation in occurrence and biomass of rooted macrophytes and drift algae in shallow bays. *Estuar Coast Shelf Sci* 56:1167-1175
- Berner D, Blanckenhorn WU, Koerner C (2005) Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos* 111:525-533
- Blamey LK, Branch GM (2012) Regime shift of a kelp-forest benthic community induced by an 'invasion' of the rock lobster *Jasus lalandii*. *Journal of Experimental Marine Biology and Ecology* 420:33-47
- Boesch D Diversity, stability and response to human disturbance in estuarine ecosystems. *Proc Proceedings of the first international congress of ecology Pudoc, Wageningen*
- Bokn TL, Duarte CM, Pedersen MF, Marba N, Moy FE, Barron C, Bjerkeng B, Borum J, Christie H, Engelbert S, Fotel FL, Hoell EE, Karez R, Kersting K, Kraufvelin P, Lindblad C, Olsen M, Sanderud KA, Sommer U, Sorensen K (2003) The response of experimental rocky shore communities to nutrient additions. *Ecosystems* 6:577-594
- Bonaviri C, Fernandez TV, Fanelli G, Badalamenti F, Gianguzza P (2011) Leading role of the sea urchin *Arbacia lixula* in maintaining the barren state in southwestern Mediterranean. *Marine Biology* 158:2505-2513
- Boyd PW, Hutchins DA (2012) Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. *Mar Ecol-Prog Ser* 470:125-135
- Brandt M, Witman JD, Chiriboga AI (2012) Influence of a dominant consumer species reverses at increased diversity. *Ecology* 93:868-878
- Bulleri F (2013) Grazing by sea urchins at the margins of barren patches on Mediterranean rocky reefs. *Marine Biology* 160:2493-2501

- Burkepile DE, Hay ME (2006) Herbivore vs nutrient control of marine primary producers: Context-dependent effects. *Ecology* 87:3128-3139
- Campbell SJ (1999) Uptake of ammonium by four species of macroalgae in Port Phillip Bay, Victoria, Australia. *Mar Freshw Res* 50:515-522
- Campbell SJ, Burrige TR (1998) Occurrence of *Undaria pinnatifida* (Phaeophyta : Laminariales) in Port Phillip Bay, Victoria, Australia. *Mar Freshw Res* 49:379-381
- Carnell PE, Keough MJ (2014) Spatially variable synergistic effects of disturbance and additional nutrients on kelp recruitment and recovery. *Oecologia* 175:409-416
- Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol Appl* 8:559-568
- Chapman A, Craigie J (1977) Seasonal growth in *Laminaria longicuris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology* 40:197-205
- Chapman ARO (1981) Stability of sea-urchin dominated barren grounds following destructive grazing of kelp in St. Margarets Bay, eastern Canada. *Marine Biology* 62:307-311
- Chapman ARO, Johnson CR (1990) Disturbance and organization of macroalgal assemblages in the Northwest Atlantic. *Hydrobiologia* 192:77-121
- Chapman AS, Fletcher RL (2002) Differential effects of sediments on survival and growth of *Fucus serratus* embryos (Fucales, Phaeophyceae) *Journal of Phycology* 38:894-903
- Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. *Mar Ecol-Prog Ser* 210:223-253
- Collie JS, Richardson K, Steele JH (2004) Regime shifts: can ecological theory illuminate the mechanisms? *Progress in Oceanography* 60:281-302
- Connell SD (2003) The monopolization of understorey habitat by subtidal encrusting coralline algae: a test of the combined effects of canopy-mediated light and sedimentation. *Marine Biology* 142:1065-1071
- Connell SD (2005) Assembly and maintenance of subtidal habitat heterogeneity: synergistic effects of light penetration and sedimentation. *Marine Ecology-Progress Series* 289:53-61
- Connell SD, Irving AD (2008) Integrating ecology with biogeography using landscape characteristics: a case study of subtidal habitat across continental Australia. *J Biogeogr* 35:1608-1621

- Connell SD, Russell BD, Turner DJ, Shepherd SA, Kildea T, Miller D, Airolidi L, Cheshire A (2008) Recovering a lost baseline: missing kelp forests from a metropolitan coast. *Marine Ecology-Progress Series* 360:63-72
- Constable AJ (1989) An investigation of resource allocation in the sea urchin *Heliocidaris erythrogramma* (Valenciennes 1846). PhD, University of Melbourne, Melbourne
- Contreras S, Castilla J (1987) Feeding behavior and morphological adaptations in two sympatric sea urchin species in central Chile. *Mar Ecol-Prog Ser* 38:217-224
- Conversi A, Dakos V, Gardmark A, Ling S, Folke C, Mumby PJ, Greene C, Edwards M, Blenckner T, Casini M, Pershing A, Mollmann C (2015) A holistic view of marine regime shifts. *Philos Trans R Soc B-Biol Sci* 370:8
- Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol Lett* 11:1304-1315
- Crawley MJ (1983) Herbivory. The dynamics of animal-plant interactions. Blackwell Scientific Publications, Oxford
- Cubit JD (1984) Herbivory and the seasonal abundance of algae on a high intertidal rocky shore. *Ecology* 65:1904-1917
- Currie DR, Parry GD (1999) Changes to benthic communities over 20 years in Port Phillip Bay, Victoria, Australia. *Marine Pollution Bulletin* 38:36-43
- Cyrus M, Bolton J, Scholtz R, Macey B (2015) The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: effects on palatability, consumption and digestibility. *Aquaculture Nutrition* 21:578-591
- Day EG, Branch GM (2002) Influences of the sea urchin *Parechinus angulosus* (Leske) on the feeding behaviour and activity rhythms of juveniles of the South African abalone *Haliotis midae* Linn. *Journal of Experimental Marine Biology and Ecology* 276:1-17
- Dayton PK (1985) Ecology of kelp communities. *Annu Rev Ecol Syst* 16:215-245
- Dayton PK, Tegner MJ (1984) Catastrophic storms, El Nino, and patch stability in a southern california kelp community. *Science* 224:283-285
- de Bettignies T, Wernberg T, Lavery PS, Vanderklift MA, Gunson JR, Symonds G, Collier N (2015) Phenological decoupling of mortality from wave forcing in kelp beds. *Ecology* 96:850-861
- Dean TA, Schroeter SC, Dixon JD (1984) Effects of grazing by two species of sea urchins (*Strongylocentrotus franciscanus* and *Lytechinus anamesus*) on recruitment and survival of two species of kelp (*Macrocystis pyrifera* and *Pterygophora californica*). *Marine Biology* 78:301-313

- Dong Q, McCormick PV, Sklar FH, DeAngelis DL (2002) Structural instability, multiple stable states, and hysteresis in periphyton driven by phosphorus enrichment in the Everglades. *Theoretical Population Biology* 61:1-13
- Duarte CM (1995) Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41:87-112
- Duggins DO (1981) Sea urchins and kelp: the effects of short term changes in urchin diet. *Limnol Oceanogr* 26:391-394
- Dumont CP, Himmelman JH, Robinson SMC (2007) Random movement pattern of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology* 340:80-89
- Edgar GJ (1997) *Australian Marine Life*. REED BOOKS, Hong Kong
- Elsdon TS, Limburg KE (2008) Nutrients and their duration of enrichment influence periphyton cover and biomass in rural and urban streams. *Mar Freshw Res* 59:467-476
- Eriksson BK, Johansson G (2005) Effects of sedimentation on macroalgae: species-specific responses are related to reproductive traits. *Oecologia* 143:438-448
- Eriksson BK, Johansson G, Snoeijis P (2002) Long-term changes in the macroalgal vegetation of the inner Gullmar Fjord, Swedish Skagerrak coast. *Journal of Phycology* 38:284-296
- Estes JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* 282:473-476
- Fabricius K, Klumberschledl A, Harrington L, Noonan S, De'Ath G (2015) In situ changes of tropical crustose coralline algae along carbon dioxide gradients. *Scientific reports* 5:9537
- Falkenberg LJ, Connell SD, Coffee OI, Ghedini G, Russell BD (2015) Species interactions can maintain resistance of subtidal algal habitats to an increasingly modified world. *Global Ecology and Conservation* 4:549-558
- Fauchald P (2010) Predator-prey reversal: A possible mechanism for ecosystem hysteresis in the North Sea? *Ecology* 91:2191-2197
- Feehan C, Scheibling RE, Lauzon-Guay JS (2012) Aggregative feeding behavior in sea urchins leads to destructive grazing in a Nova Scotian kelp bed. *Mar Ecol-Prog Ser* 444:69-83

- Fehmi JS, Karn JF, Ries RE, Hendrickson JR, Hanson JD (2002) Cattle grazing behavior with season-long free-choice access to four forage types. *Applied Animal Behaviour Science* 78:29-42
- Filbee-Dexter K, Scheibling RE (2014) Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Mar Ecol-Prog Ser* 495:1-25
- Flukes E, Johnson C, Wright J (2014) Thinning of kelp canopy modifies understory assemblages: the importance of canopy density. *Mar Ecol Prog Ser* 514:57-70
- Flukes EB, Johnson CR, Ling SD (2012) Forming sea urchin barrens from the inside out: an alternative pattern of overgrazing. *Mar Ecol-Prog Ser* 464:179-194
- Fox R, Bellwood D (2013) Niche partitioning of feeding microhabitats produces a unique function for herbivorous rabbitfishes (Perciformes, Siganidae) on coral reefs. *Coral Reefs* 32:13-23
- Fulton B, Smith T (2002) Ecosim Case Study: Port Phillip Bay, Australia. Fisheries Centre Research Reports 10:83
- Fung T, Seymour RM, Johnson CR (2011) Alternative stable states and phase shifts in coral reefs under anthropogenic stress. *Ecology* 92:967-982
- Gagnon P, Himmelman JH, Johnson LE (2004) Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. *Marine Biology* 144:1191-1203
- Gorgula SK, Connell SD (2004) Expansive covers of turf-forming algae on human-dominated coast: the relative effects of increasing nutrient and sediment loads. *Marine Biology* 145
- Gorman D, Russell BD, Connell SD (2009) Land-to-sea connectivity: linking human-derived terrestrial subsidies to subtidal habitat change on open rocky coasts. *Ecol Appl* 19:1114-1126
- Guarnieri G, Bevilacqua S, Vignes F, Fraschetti S (2014) Grazer removal and nutrient enrichment as recovery enhancers for overexploited rocky subtidal habitats. *Oecologia* 175:959-970
- Guerry AD, Menge BA, Dunmore RA (2009) Effects of consumers and enrichment on abundance and diversity of benthic algae in a rocky intertidal community. *Journal of Experimental Marine Biology and Ecology* 369:155-164
- Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. *American Naturalist* 94:421-425

- Hanski I, Korpimäki E (1995) Microtine rodent dynamics in northern Europe: parameterized models for the predator-prey interaction. *Ecology* 76:840-850
- Hansson L (1977) Landscape ecology and stability of populations. *Landscape Planning* 4:85-93
- Harding APC, Scheibling RE (2015) Feed or flee: Effect of a predation-risk cue on sea urchin foraging activity. *Journal of Experimental Marine Biology and Ecology* 466:59-69
- Harris G, Batley G, Fox D, Hall D, Jernakoff P, Molloy R, Murray A, Newell B, Parslow J, Skyring G, Walker S (1996) Port Phillip Bay Environmental study Final Report. CSIRO Publishing, Collingwood, Vic
- Harris GP, Crossland CJ (1999) Introduction to the Port Phillip Bay environmental study. *Mar Freshw Res* 50:III-IV
- Harrold C, Light K, Lisin S (1998) Organic enrichment of submarine-canyon and continental-shelf benthic communities by macroalgal drift imported from nearshore kelp forests. *Limnol Oceanogr* 43:669-678
- Harrold C, Reed DC (1985) Food availability, sea-urchin grazing, and kelp forest community structure *Ecology* 66:1160-1169
- Hauxwell J, McClelland J, Behr PJ, Valiela I (1998) Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. *Estuaries* 21:347-360
- Heggie DT, Skyring GW, Orchardo J, Longmore AR, Nicholson GJ, Berelson WM (1999) Denitrification and denitrifying efficiencies in sediments of Port Phillip Bay: direct determinations of biogenic N(2) and N-metabolite fluxes with implications for water quality. *Mar Freshw Res* 50:589-596
- Hewitt CL, Campbell ML, Thresher RE, Martin RB, Boyd S, Cohen BF, Currie DR, Gomon MF, Keough MJ, Lewis JA, Lockett MM, Mays N, McArthur MA, O'Hara TD, Poore GCB, Ross DJ, Storey MJ, Watson JE, Wilson RS (2004) Introduced and cryptogenic species in Port Phillip Bay, Victoria, Australia. *Marine Biology* 144:183-202
- Hiddink JG, ter Hofstede R (2008) Climate induced increases in species richness of marine fishes. *Global Change Biology* 14:453-460
- Hill NA, Blount C, Poore AGB, Worthington D, Steinberg PD (2003) Grazing effects of the sea urchin *Centrostephanus rodgersii* in two contrasting rocky reef habitats: effects of urchin density and its implications for the fishery. *Mar Freshw Res* 54:691-700
- Hillebrand H (2003) Opposing effects of grazing and nutrients on diversity. *Oikos* 100:592-600

- Hills J, Thomason J (1998) The effect of scales of surface roughness on the settlement of barnacle (*Semibalanus balanoides*) cyprids. *Biofouling* 12:57-69
- Hirota M, Holmgren M, Van Nes EH, Scheffer M (2011) Global resilience of tropical forest and savanna to critical transitions. *Science* 334:232-235
- Hobday AJ, Tegner MJ, Haaker PL (2000) Over-exploitation of a broadcast spawning marine invertebrate: decline of the white abalone. *Reviews in Fish Biology and Fisheries* 10:493-514
- Holling CS (1973) Resilience and Stability of Ecological Systems. *Annu Rev Ecol Syst* 4:1-23
- Holling CS (1996) Engineering resilience versus ecological resilience. In: Schulze PC (ed) *Engineering Within Ecological Constraints*. National Academy Press, Washington, DC
- Hughes TP, Linares C, Dakos V, van de Leemput IA, van Nes EH (2013) Living dangerously on borrowed time during slow, unrecognized regime shifts. *Trends in Ecology & Evolution* 28:149-155
- Hunter MD, Price PW (1992a) Playing chutes and ladders - heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724-732
- Hunter MD, Price PW (1992b) Playing chutes and ladders heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology (Washington D C)* 73:724-732
- Irving AD, Balata D, Colosio F, Ferrando GA, Airoidi L (2009) Light, sediment, temperature, and the early life-history of the habitat-forming alga *Cystoseira barbata*. *Marine Biology* 156:1223-1231
- Isaeus M, Malm T, Persson S, Svensson A (2004) Effects of filamentous algae and sediment on recruitment and survival of *Fucus serratus* (Phaeophyceae) juveniles in the eutrophic Baltic Sea. *Eur J Phycol* 39:301-307
- Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629-638
- Jenkins GP (2004) The ecosystem effects of abalone fishing: a review. *Mar Freshw Res* 55:545-552
- Johnson C, Valentine J, Pederson H A most unusual barrens: complex interactions between lobsters, sea urchins and algae facilitates spread of an exotic kelp in eastern Tasmania.

- Proc Echinoderms: Munchen: Proceedings of the 11th International Echinoderm Conference, 6-10 October 2003, Munich, Germany. CRC Press
- Johnson CR, Mann KH (1988) Diversity, patterns of adaptation, and stability of Nova Scotian kelp beds. *Ecol Monogr* 58:129-154
- Johnson TR, Wilson JA, Cleaver C, Morehead G, Vadas R (2013) Modeling fine scale urchin and kelp dynamics: Implications for management of the Maine sea urchin fishery. *Fisheries Research* 141:107-117
- Jung CA, Dwyer PD, Minnegal M, Swearer SE (2011) Perceptions of environmental change over more than six decades in two groups of people interacting with the environment of Port Phillip Bay, Australia. *Ocean & Coastal Management* 54:93-99
- Karez R, Engelbert S, Kraufvelin P, Pedersen MF, Sommer U (2004) Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. *Aquatic Botany* 78:103-117
- Kawamata S, Yoshimitsu S, Tanaka T, Igari T, Tokunaga S (2011) Importance of sedimentation for survival of canopy-forming fucoid algae in urchin barrens. *Journal of Sea Research* 66:76-86
- Kawamata S, Yoshimitsu S, Tokunaga S, Kubo S, Tanaka T (2012) Sediment tolerance of Sargassum algae inhabiting sediment-covered rocky reefs. *Marine Biology* 159:723-733
- Kennelly SJ (1987) Physical disturbances in an Australian kelp community, 1. Temporal effects. *Mar Ecol-Prog Ser* 40:145-153
- Kennelly SJ (1989) Effects of kelp canopies on understory species due to shade and scour. *Mar Ecol-Prog Ser* 50:215-224
- Kirkman H (1984) Standing stock and production of *Ecklonia radiata* (C. Ag.): J. Agardh. *Journal of Experimental Marine Biology and Ecology* 76:119-130
- Konar B (2000) Seasonal inhibitory effects of marine plants on sea urchins: structuring communities the algal way. *Oecologia* 125:208-217
- Konar B (2001) Seasonal changes in subarctic sea urchin populations from different habitats. *Polar Biology* 24:754-763
- Konar B, Estes JA (2003) The stability of boundary regions between kelp beds and deforested areas. *Ecology* 84:174-185
- Korpinen S, Jormalainen V (2008) Grazing and nutrients reduce recruitment success of *Fucus vesiculosus* L. (Fucales : Phaeophyceae). *Estuar Coast Shelf Sci* 78:437-444

- Korpinen S, Jormalainen V, Honkanen T (2007) Effects of nutrients, herbivory, and depth on the macroalgal community in the rocky sublittoral. *Ecology* 88:839-852
- Kraufvelin P (1999) Baltic hard bottom mesocosms unplugged: replicability, repeatability and ecological realism examined by non-parametric multivariate techniques. *Journal of Experimental Marine Biology and Ecology* 240:229-258
- Kraufvelin P, Lindholm A, Pedersen MF, Kirkerud LA, Bonsdorff E (2009) Biomass, diversity and production of rocky shore macroalgae at two nutrient enrichment and wave action levels. *Marine Biology* 157:29-47
- Kraufvelin P, Lindholm A, Pedersen MF, Kirkerud LA, Bonsdorff E (2010) Biomass, diversity and production of rocky shore macroalgae at two nutrient enrichment and wave action levels. *Marine Biology* 157:29-47
- Kriegisch N, Reeves S, Johnson CR, Ling SD (2016) Phase-Shift Dynamics of Sea Urchin Overgrazing on Nutrified Reefs. *PloS one* 11:e0168333
- Lalli CMe (1990) *Enclosed Experimental Marine Ecosystems: A Review and Recommendations*, Vol 37. Springer-Verlag, New York
- Last PR, White WT, Gledhill DC, Hobday AJ, Brown R, Edgar GJ, Pecl G (2011) Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography* 20:58-72
- Lauzon-Guay J-S, Scheibling RE (2007) Behaviour of sea urchin *Strongylocentrotus droebachiensis* grazing fronts: food-mediated aggregation and density-dependent facilitation. *Mar Ecol-Prog Ser* 329:191-204
- Lauzon-Guay JS, Scheibling RE, Barbeau MA (2006) Movement patterns in the green sea urchin, *Strongylocentrotus droebachaensis*. *J Mar Biol Assoc UK* 86:167-174
- Lawrence J. M (1975) On the relationships between marine plants and sea urchins. *Oceanogr Mar Biol Ann Rev* 13:213-286
- Lindenmayer DB, Hobbs RJ, Likens GE, Krebs CJ, Banks SC (2011) Newly discovered landscape traps produce regime shifts in wet forests. *Proceedings of the National Academy of Sciences of the United States of America* 108:15887-15891
- Ling SD (2008) Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. *Oecologia* 156:883-894
- Ling SD, Ibbott S, Sanderson JC (2010) Recovery of canopy-forming macroalgae following removal of the enigmatic grazing sea urchin *Heliocidaris erythrogramma*. *Journal of Experimental Marine Biology and Ecology* 395:135-146

- Ling SD, Johnson CR (2012) Marine reserves reduce risk of climate-driven phase shift by reinstating size- and habitat-specific trophic interactions. *Ecol Appl* 22:1232-1245
- Ling SD, Johnson CR, Frusher SD, Ridgway KR (2009a) Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences of the United States of America* 106:22341-22345
- Ling SD, Johnson CR, Ridgway K, Hobday AJ, Haddon M (2009b) Climate-driven range extension of the sea urchin: inferring future trends by analysis of recent population dynamics. *Global Change Biology* 15:719-731
- Ling SD, Scheibling RE, Rassweiler A, Johnson CR, Shears N, Connell SD, Salomon AK, Norderhaug KM, Pérez-Matus A, Hernández JC, Clemente S, Blamey LK, Hereu B, Ballesteros E, Sala E, Garrabou J, Cebrian E, Zabala M, Fujita D, Johnson LE (2015) Global regime shift dynamics of catastrophic sea urchin overgrazing, Vol 370
- Lobban CS, Harrison PJ (1994) *Seaweed ecology and physiology*. Cambridge University Press
- Lotze HK, Worm B, Sommer U (2001) Strong bottom-up and top-down control of early life stages of macroalgae. *Limnol Oceanogr* 46:749-757
- Lubchenco J, Gaines SD (1981) A unified approach to marine plant-herbivore interactions. I. Populations and communities. *Annu Rev Ecol Syst*:405-437
- Lundberg B, Golani D (1995) Diet Adaptations of *Lessepsian* Migrant Rabbitfishes, *Siganus luridus* and *S. rivulatus*, to the Algal Resources of the Mediterranean Coast of Israel. *Marine Ecology* 16:73-89
- Martinetto P, Teichberg M, Valiela I, Montemayor D, Iribarne O (2011) Top-down and bottom-up regulation in a high nutrient-high herbivory coastal ecosystem. *Mar Ecol-Prog Ser* 432:69-82
- Marzloff MP, Dambacher JM, Johnson CR, Little LR, Frusher SD (2011) Exploring alternative states in ecological systems with a qualitative analysis of community feedback. *Ecological Modelling* 222:2651-2662
- Mattison JE, Trent JD, Shanks AL, Akin TB, Pearse JS (1977) Movement and feeding activity of red sea urchins (*Strongylocentrotus franciscanus*) adjacent to a kelp forest. *Marine Biology* 39:25-30
- May RM (1977) Thresholds and breaking points in ecosystems with a multiplicity of stable states. *Nature* 269:471-477
- Mearns AJ, Reish DJ, Oshida PS, Ginn T, Rempel-Hester MA (2011) Effects of Pollution on Marine Organisms. *Water Environ Res* 83:1789-1852

- Menge BA (2000) Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology* 250:257-289
- Menge BA, Sutherland JP (1976) Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. *American naturalist*:351-369
- Miller LP, Gaylord B (2007) Barriers to flow: the effects of experimental cage structures on water velocities in high-energy subtidal and intertidal environments. *Journal of Experimental Marine Biology and Ecology* 344:215-228
- Muthukrishnan R, Fong P (2014) Multiple anthropogenic stressors exert complex, interactive effects on a coral reef community. *Coral Reefs* 33:911-921
- Nearing M, Pruski F, O'neal M (2004) Expected climate change impacts on soil erosion rates: a review. *Journal of Soil and Water Conservation* 59:43-50
- Newcombe EM, Cardenas CA, Geange SW (2012) Green sea urchins structure invertebrate and macroalgal communities in the Magellan Strait, southern Chile. *Aquat Biol* 15:135-U199
- Nixon SW (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* 41:199-219
- Noriega R, Schlacher TA, Smeuninx B (2012) Reductions in Ghost Crab Populations Reflect Urbanization of Beaches and Dunes. *Journal of Coastal Research* 28:123-131
- North W, Zimmerman R Influences of macronutrients and water temperatures on summertime survival of *Macrocystis* canopies. *Proc Eleventh International Seaweed Symposium*. Springer
- Ogden JC, Lobel PS (1978) The role of herbivorous fishes and urchins in coral reef communities. *Environmental biology of fishes* 3:49-63
- Ortega-Borges L, Tuya F, Haroun RJ (2009) Does depth and sedimentation interact with sea urchins to affect algal assemblage patterns on eastern Atlantic reefs? *Journal of Shellfish Research* 28:947-955
- Osman RW, Munguia P, Zajac RN (2010) Ecological thresholds in marine communities: theory, experiments and management. *Mar Ecol-Prog Ser* 413:185-187
- Paine RT, Tegner MJ, Johnson EA (1998) Compounded perturbations yield ecological surprises. *Ecosystems* 1:535-545
- Pederson HG, Johnson CR (2006) Predation of the sea urchin *Heliocidaris erythrogramma* by rock lobsters (*Jasus edwardsii*) in no-take marine reserves. *Journal of Experimental Marine Biology and Ecology* 336:120-134

- Peterson G, Allen CR, Holling CS (1998) Ecological resilience, biodiversity, and scale. *Ecosystems* 1:6-18
- Pfister CA, Van Alstyne KL (2003) An experimental assessment of the effects of nutrient enhancement on the intertidal kelp *Hedophyllum sessile* (Laminariales, Phaeophyceae). *Journal of Phycology* 39:285-290
- Piazzzi L, Ceccherelli G (2006) Persistence of biological invasion effects: Recovery of macroalgal assemblages after removal of *Caulerpa racemosa* var. *cylindracea*. *Estuarine Coastal and Shelf Science* 68:455-461
- Pimm SL (1984) The complexity and stability of ecosystems. *Nature* 307:321-326
- Pinnegar JK, Polunin NVC, Francour P, Badalamenti F, Chemello R, Harmelin-Vivien ML, Hereu B, Milazzo M, Zabala M, D'Anna G, Pipitone C (2000) Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. *Environmental Conservation* 27:179-200
- Poore AGB, Campbell AH, Coleman RA, Edgar GJ, Jormalainen V, Reynolds PL, Sotka EE, Stachowicz JJ, Taylor RB, Vanderklift MA, Duffy JE (2012) Global patterns in the impact of marine herbivores on benthic primary producers. *Ecol Lett* 15:912-922
- Posey MH, Alphin TD, Cahoon LB, Lindquist DG, Mallin MA, Nevers MB (2002) Top-down versus bottom-up limitation in benthic infaunal communities: Direct and indirect effects. *Estuaries* 25:999-1014
- Power ME (1992) Top-down and bottom-up forces in food webs: do plants have primacy. *Ecology* 73:733-746
- Preen A (1995) Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. *Marine ecology progress series* Oldendorf 124:201-213
- Rockstroem J, Steffen W, Noone K, Persson A, Chaplin FS, Lambin EF (2009) A safe operating space for humanity. *Nature* 461:472-475
- Rodriguez SR, Farina JM (2001) Effect of drift kelp on the spatial distribution pattern of the sea urchin *Tetrapygus niger*: a geostatistical approach. *J Mar Biol Assoc UK* 81:179-180
- Rosenzweig ML, MacArthur RH (1963) Graphical representation and stability conditions of predator-prey interactions. *The American Naturalist* 97:209-223
- Russell BD (2007) Effects of canopy-mediated abrasion and water flow on the early colonisation of turf-forming algae. *Mar Freshw Res* 58:657-665

- Russell BD, Connell SD (2005) A novel interaction between nutrients and grazers alters relative dominance of marine habitats. *Marine Ecology-Progress Series* 289:5-11
- Russell BD, Elsdon TS, Gillanders BM, Connell SD (2005) Nutrients increase epiphyte loads: broad-scale observations and an experimental assessment. *Marine Biology* 147:551-558
- Russell BD, Thompson JAI, Falkenberg LJ, Connell SD (2009) Synergistic effects of climate change and local stressors: CO₂ and nutrient-driven change in subtidal rocky habitats. *Global Change Biology* 15:2153-2162
- Russo AR (1979) Dispersion and food differences between two populations of the sea urchin *Strongylocentrotus franciscanus*. *J Biogeogr*:407-414
- Sala E, Boudouresque CF, Harmelin-Vivien M (1998) Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. *Oikos* 82:425-439
- Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. *Nature* 413:591-596
- Scheffer M, Carpenter SR (2003a) Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology & Evolution* 18:648-656
- Scheffer M, Carpenter SR (2003b) Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology & Evolution* 18:648-656
- Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E (1993) Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution* 8:275-279
- Scheibling RE, Hennigar AW, Balch T (1999) Destructive grazing, epiphytism, and disease: the dynamics of sea urchin - kelp interactions in Nova Scotia. *Can J Fish Aquat Sci* 56:2300-2314
- Schiel DR, Foster MS (1986) The structure of subtidal algal stands in temperate waters. *Oceanography and Marine Biology* 24:265-307
- Shears NT, Babcock RC (2002) Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia* 132:131-142
- Shepherd SA, Watson JE, Womersley HBS, Carey JM (2009) Long-term changes in macroalgal assemblages after increased sedimentation and turbidity in Western Port, Victoria, Australia. *Botanica Marina* 52:195-206
- Sjoo GL, Mork E, Andersson S, Melander I (2011) Differences in top-down and bottom-up regulation of macroalgal communities between a reef crest and back reef habitat in Zanzibar. *Estuar Coast Shelf Sci* 91:511-518

- Smith J, Smith C, Hunter C (2001) An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. *Coral Reefs* 19:332-342
- Smith JE, Hunter CL, Smith CM (2010) The effects of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia* 163:497-507
- Sokos C, Andreadis K, Papageorgiou N (2015) Diet adaptability by a generalist herbivore: the case of brown hare in a Mediterranean agroecosystem. *Zoological Studies* 54:27
- Steinberg PD (1995) Sasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. *Oecologia* 102:169-173
- Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* 29:436-459
- Sternberg LDL (2001) Savanna-forest hysteresis in the tropics. *Global Ecology and Biogeography* 10:369-378
- Strain EMA, Thomson RJ, Micheli F, Mancuso FP, Airolidi L (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*
- Tegner MJ, Dayton PK (2000) Ecosystem effects of fishing in kelp forest communities. *ICES J Mar Sci* 57:579-589
- Tennant JP, MacLeod N (2014) Snout shape in extant ruminants. *PloS one* 9:e112035
- Terradas J, Penuelas J (2011) Misleading ideas about top-down and bottom-up control in communities and the role of omnivores. *Polish Journal of Ecology* 59:849-850
- Tilman D, Downing JA (1994) Biodiversity and stability in grasslands. *Nature* 367:363-365
- Umar M, McCook L, Price I (1998) Effects of sediment deposition on the seaweed *Sargassum* on a fringing coral reef. *Coral Reefs* 17:169-177
- Vadas R, Elner R, Garwood P, Babb I (1986) Experimental evaluation of aggregation behavior in the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* 90:433-448
- Valentine JP, Johnson CR (2003) Establishment of the introduced kelp *Undaria pinnatifida* in Tasmania depends on disturbance to native algal assemblages. *Journal of Experimental Marine Biology and Ecology* 295:63-90
- Valentine JP, Johnson CR (2005a) Persistence of sea urchin (*Heliocidaris erythrogramma*) barrens on the east coast of Tasmania: inhibition of macroalgal recovery in the absence of high densities of sea urchins. *Botanica Marina* 48:106-115

- Valentine JP, Johnson CR (2005b) Persistence of the exotic kelp *Undaria pinnatifida* does not depend on sea urchin grazing. *Mar Ecol-Prog Ser* 285:43-55
- Van Alstyne KL, Pelletreau KN (2000) Effects of nutrient enrichment on growth and phlorotannin production in *Fucus gardneri* embryos. *Mar Ecol Prog Ser* 206
- Vanderklift MA, Kendrick GA (2004) Variation in abundances of herbivorous invertebrates in temperate subtidal rocky reef habitats. *Mar Freshw Res* 55:93-103
- Vanderklift MA, Kendrick GA (2005) Contrasting influence of sea urchins on attached and drift macroalgae. *Marine Ecology-Progress Series* 299:101-110
- Vanderklift MA, Lavery PS, Waddington KI (2009) Intensity of herbivory on kelp by fish and sea urchins differs between inshore and offshore reefs. *Marine Ecology-Progress Series* 376:203-211
- Vanderklift MA, Wernberg T (2008) Detached kelps from distant sources are a food subsidy for sea urchins. *Oecologia* 157:327-335
- Villouta E, Chadderton WL, Pugsley CW, Hay CH (2001) Effects of sea urchin (*Evechinus chloroticus*) grazing in Dusky Sound, Fiordland, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 35:1007-1024
- Vinueza LR, Menge BA, Ruiz D, Palacios DM (2014) Oceanographic and climatic variation drive top-down/bottom-up coupling in the Galapagos intertidal meta-ecosystem. *Ecol Monogr* 84:411-434
- Walker B, Holling CS, Carpenter SR, Kinzig A (2004) Resilience, adaptability and transformability in social-ecological systems. *Ecology and Society* 9
- Walker BH, Ludwig D, Holling CS, Peterman RM (1981) Stability of semi-arid savanna grazing systems. *The Journal of Ecology*:473-498
- Williams D, Anderson D (1975) The reproductive system, embryonic development, larval development and metamorphosis of the sea urchin *Heliocidaris erythrogramma* (Val.)(Echinoidea: Echinometridae). *Australian Journal of Zoology* 23:371-403
- Worm B, Lotze HK (2006) Effects of eutrophication, grazing, and algal blooms on rocky shores. *Limnol Oceanogr* 51:569-579
- Worm B, Lotze HK, Bostroem C, Engkvist R, Labanauskas V, Sommer U (1999) Marine diversity shift linked to interactions among grazers, nutrients and propagule banks. *Mar Ecol Prog Ser* 185:309-314
- Worm B, Lotze HK, Hillebrand H, Sommer U (2002) Consumer *versus* resource control of species diversity and ecosystem functioning. *Nature* 417:848-851

- Worm B, Reusch TBH, Lotze HK (2000) *In situ* nutrient enrichment: Methods for marine benthic ecology. International Review of Hydrobiology 85:359-375
- Wright JT, Dworjanyn SA, Rogers CN, Steinberg PD, Williamson JE, Poore AGB (2005) Density-dependent sea urchin grazing: differential removal of species, changes in community composition and alternative community states. Marine Ecology-Progress Series 298:143-156

Appendix

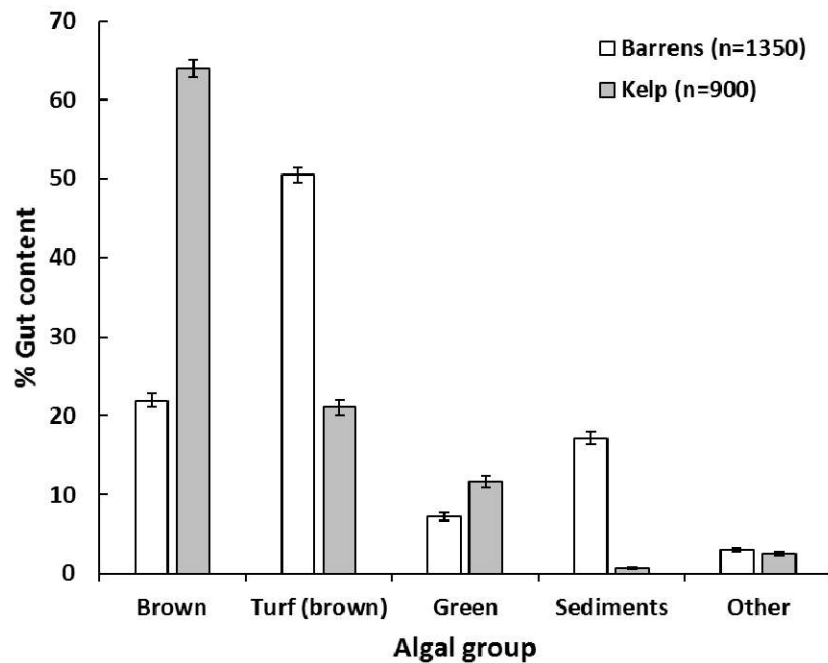


Figure 1: Composition of gut contents for *Heliocidaris erythrogramma* for kelp bed and barrens habitat for Port Phillip Bay. Data has been pooled for 3 regions of Port Phillip Bay (west, north and south east). Sea urchins were collected and brought to the laboratory where the percentage composition of guts contents was by assessing the planar percentage cover of the contents spread evenly across a petri dish. The content was scored in categories of brown algae (Brown), filamentous algae mixed with sediments (Turf), green algae (Green), sediments and shell material (Sediments) or unidentifiable material (Other).